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EFFECT OF HORMONE PRETREATMENT ON CONTRACTILITY OF RABBIT OVIDUCTAL ISTHMUS : ROLE OF ADRENERGIC MECHANISMS

by

(C) DORIANNE E. RHEAUME

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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EDMONTON, ALBERTA

(FALL, 1977)

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Effect of Hormone Pre-treatment on Contractility of Rabbit Oviductal Isthmus: Role of Adrenergic Mechanisms", submitted by Dorianne E. Rheaume in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date ..August...23,.1977...

ABSTRACT

The exact mechanism for the hormonal modification of ovum transport is not known. The effects of transmural stimulation and of adrenergic agonists on the contractility of isthmic circular muscle of the oviduct were investigated in rabbits in estrus and after hormone treatments which disrupt normal ovum transport. We studied certain oviductal adrenergic mechanisms to define their possible role in mediating hormonal control over oviduct function.

Stimulation at 24 Hz was maximal in all tissues but the tension developed was greater in the Estrous group than in the HCG-treated groups. Also, the frequency required to produce a 50% of maximum response was less in Estrous tissues. Cocaine, hydrocortisone and propranolol did not eliminate these differences.

Both α- and β-adrenoceptors were present in all tissues. The α-receptors were predominant in all cases; transmural stimulation, adrenaline and noradrenaline always caused contractions that were blocked by phenoxybenzamine.

(-)-Adrenaline was more potent than (-)-noradrenaline or (-)-phenylephrine in all tissues. Agonist potency between groups did not vary but for one notable exception: in HCG + Progesterone tissues, noradrenaline was significantly less potent than in the Estrous or HCG + Estrogen groups.

Modifications in catecholamine reuptake processes and α- and β-adrenoceptor sensitivity changes were investigated as possible explanations for these observations. Neither cocaine (3×10^{-5}M) nor cocaine + hydrocortisone (10^{-4}M) eliminated the between-group noradrenaline potency differences. α-Adrenoceptor sensitivity did not vary between groups, since the potencies of phenylephrine in the presence of propranolol and of adrenaline in the

presence of cocaine + hydrocortisone + propranolol were not affected by hormone treatment. Propranolol (3.8×10^{-6}M) potentiated noradrenaline only in the HCG + Progesterone group and abolished between-group noradrenaline potency differences. Therefore, the possibility of increased β-activity in HCG + Progesterone tissues was further investigated.

We studied β-activity by measuring the ability of adrenergic agonists to inhibit contractile responses to acetylcholine in the presence of cocaine + hydrocortisone + pre-exposure to phenoxybenzamine. (±)-Isoprenaline inhibited the acetylcholine response by 90% in HCG + Progesterone tissues but by only 30% in Estrous tissues. Papaverine, however, a non-specific smooth muscle relaxant, produced identical dose-response curves in both hormone groups. ID_{50} values for (±)-isoprenaline, calculated from normalized curves, were not significantly different, however.

The order of agonist potency on β-adrenoceptors ((±)-isoprenaline ≥ (-)-noradrenaline >(-)-adrenaline >>(±)-salbutamol), as well as pA_2 values for β-antagonists, propranolol (8.3) and practolol (5.3) were similar to those quoted by Furchgott (1972) for β_1-receptors.

These results indicate that HCG + Progesterone-pretreatment causes increased β-activity possibly by increasing numbers of β-receptors, and also suggest that the β-receptor is largely β_1 in nature. Increased β-activity may be responsible for the reduced responsiveness to noradrenaline following progesterone treatment; this adrenergic phenomenon might decrease the isthmic barrier to transport and result in accelerated ovum transport.

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INTRODUCTION

I Historical Background

Although Fallopius is accredited with the first accurate description of the oviduct, its presence was realized as early as the eighth century B.C. In ancient Hindu medical writings there was reference to "two canals, the root of which is the uterus" (Woodruff and Pauerstein, 1969, p. 1).

Aristotle was aware of these uterine tubes but considered them indistinct from the uterus. Influenced by his concept of reproduction, according to which the female semen or 'catamenia' was secreted by the uterus, Aristotle assigned no function to the oviduct.

In the third century B.C., Herophilus first described the human ovaries and associated ducts. He referred to the oviducts as semen-carrying vessels and correlated them with the epididymis and vas deferens of the male reproductive system. This comparison was important since it suggested that the oviducts originated from a gonad and might function as vessels for the transfer of gametes. Unfortunately, the analogy was extended in such a direction that Herophilus designated the termination of the female conduits to be in the neck of the bladder.

It was in the first century A.D. that Rufus of Ephesus, in his description of the sheep oviduct, established that the oviducts emptied into the uterine cavity. In the next century Galen contributed to the state of knowledge by assigning a function to the oviduct. From his dissection of domestic animals, Galen described the oviducts as efferent vessels which conducted the female semen from the female testes (ovaries) to the horns of the uterus. Galen's theory was predominant throughout the medieval period and served to obscure the true identity and function of the oviduct for hundreds of years (Bodemer, 1969).

The next important contribution in the study of the mammalian oviduct was in the sixteenth century. Vesalius, still greatly influenced by Herophilus and Galen, retained the analogy to the male reproductive tract but did distinguish that the uterine tube encircled the female testes while the epididymis attached only to the posterior surface of the male testes. In 1543 he described the course of the 'semen-carrying vessel' almost correctly but failed to recognize that the oviduct was not actually attached to the ovary (Herlinger and Feiner, 1964).

Hence, Gabriele Fallopius in 1561 produced the first accurate description of the oviduct (Fallopian tube) and its anatomical relationships. He referred to the oviduct as an independent organ with a specialized structure at the abdominal end. While Fallopius had established the foundation for further understanding of oviduct function, limits to advances in this area were imposed by the traditional concepts of generation and embryogenesis.

However, in the second half of the seventeenth century, Johannis van Horne detected the presence of ova in the ovaries and noted that the Fallopian tube was hollow and communicated with the uterine cavity. He suggested that the oviduct might carry the ova to the uterus, but this theory was often disclaimed on the basis of the structural discontinuity of the oviduct and ovary.

In 1724, Dionis proposed that, by the action of ligaments, the oviduct may be brought into close contact with the ovary. Some fifty years later, Cruickshank described the fertilization of ova within the tube and studied the time course of ovum transport in the rabbit oviduct. Thus, the eighteenth century was the seat of increased interest in oviduct function. Oviduct motility received increased emphasis, and longitudinal and circular muscle fibres of the oviduct were described in this century.

By the mid-nineteenth century, knowledge of the mammalian oviduct was relatively complete: its general structure was known, the oviduct was considered the site of fertilization and early embryogenesis, ciliary and muscular mechanisms accounted for ovum transport, and the secretory function of the oviduct was recognized (Bodemer, 1969).

Thereafter, the origin of the sympathetic innervation to the female reproductive tract was described by Langley and Anderson (1894, 1895, 1896). Along with the advent of methods of studying tubal motility (kymography, tubal insufflation), this facilitated physiological analyses and pharmacological manipulations of the oviduct smooth muscle. Kok (1927) first applied adrenaline to isolated oviduct segments and monitored contractile responses. The presence of α- and β-adrenoceptors has subsequently been demonstrated in oviduct smooth muscle (Longley et al., 1968; Levy and Lindner, 1972).

Adrenergic mechanisms have been implicated in the regulation of ovum transport (Brundin, 1965), and sex hormones have been shown to affect such transport (Burdick and Pincus, 1935; Greenwald, 1961; Pauerstein et al., 1974a). Hence, interest has recently arisen in the concept that adrenergic mechanisms may play a role in mediating hormonal influences on oviduct function.

Thus, the historical background to the present study of the influence of sex hormones on adrenoceptor sensitivity in the isthmus of the rabbit oviduct is extensive and colourful. Presently, there are several books and review articles which are devoted to a comprehensive discussion of the oviduct and its function (Pauerstein et al., 1968; Hafez and Blandau, 1969; Woodruff and Pauerstein, 1969; Johnson and Foley, 1974; Pauerstein, 1974; Pauerstein et al., 1974b; Paton et al., in press). The following sections include more detailed information concerning the structure, in-

nervation, adrenoceptors, motility, and transport functions of the oviduct which is essential to a full understanding of the experimental results reported.

II Anatomy of the Oviduct

The mammalian oviduct is morphologically and functionally complex. Hence, a description of its structure and anatomical relationships at this point should enhance an understanding of its physiology and functions.

The paired oviducts extend laterally from their origin at the ovaries and terminate within the myometrium. The Fallopian tube is suspended along its length by the mesosalpinx, a derivative of the broad ligament (Nalbandov, 1969). The superior aspect of the tube is apposed to the intestinal and pelvic peritoneum. At the ovarian end the infundibulum approximates the surface of the ovary, and, at its uterine insertion, the oviduct is related to the round ligament and to the suspensory ligaments of the ovary (Woodruff and Pauerstein, 1969).

In describing the morphology of the Fallopian tube, it is convenient to consider four segments which can be distinguished anatomically. These have been defined as the pre-ampulla at the ovarian end, the ampulla, the isthmus and, finally, the junctural portion at the uterine insertion (Nilsson and Reinius, 1969).

The pre-ampulla is essentially a fimbriated, funnel-shaped structure (infundibulum) which is uniquely adapted for receiving ovulated eggs into the tube. The ampulla forms the distal half of the tube and may be distinguished from the isthmus by its larger diameter and thinner muscular walls. The ampulla is the site of fertilization of ova while the isthmus contributes to the nutrition and transport of sperm and eggs. The junctura marks the junction of the oviduct with the uterus and includes that portion

of the tube which lies within the musculature of the uterus.

The relative proportion of the tube comprised by each of the four segments and the degree of convolution of the oviducts varies between species (see Fig. 1).

The oviduct wall is composed of three distinct layers: an outer connective tissue coat (tunica serosa), a central muscular layer (tunica muscularis) and an inner mucosal layer (tunica mucosa).

The tunica serosa is a highly vascularized mesothelium which is continuous with the general peritoneum. The serosa contains irregularly distributed smooth muscle fibres which are also present in the surrounding mesentery tissues. These contractile fibres may serve some function in the contractions of the mesosalpinx which have been observed to change the position of the oviduct in the rabbit (Blandau, 1969). Such movement may be of significance for ovum pick-up during which the infundibulum is brought into close contact with the ovary so as to facilitate the entrance of ova into the tube.

The tunica muscularis is made up of circular and longitudinal smooth muscle fibres, the arrangement and thickness of which varies among species and between tubal segments. In general, there is a transitional decrease in thickness from the junctural end to the infundibulum. Beck and Boots (1974) have described four different patterns of muscle layer arrangement and have subsequently categorized oviductal segments from a number of species. For example, the ampulla and isthmus of the cat and rabbit have definite, thin layers of longitudinal muscle on either side of a thicker circular muscle layer. The human isthmus, on the other hand, has a definite circular layer with only scattered longitudinal bundles. In the human ampulla, a single, thin, but defined longitudinal layer is present exterior to a layer of circular muscle. These variations in

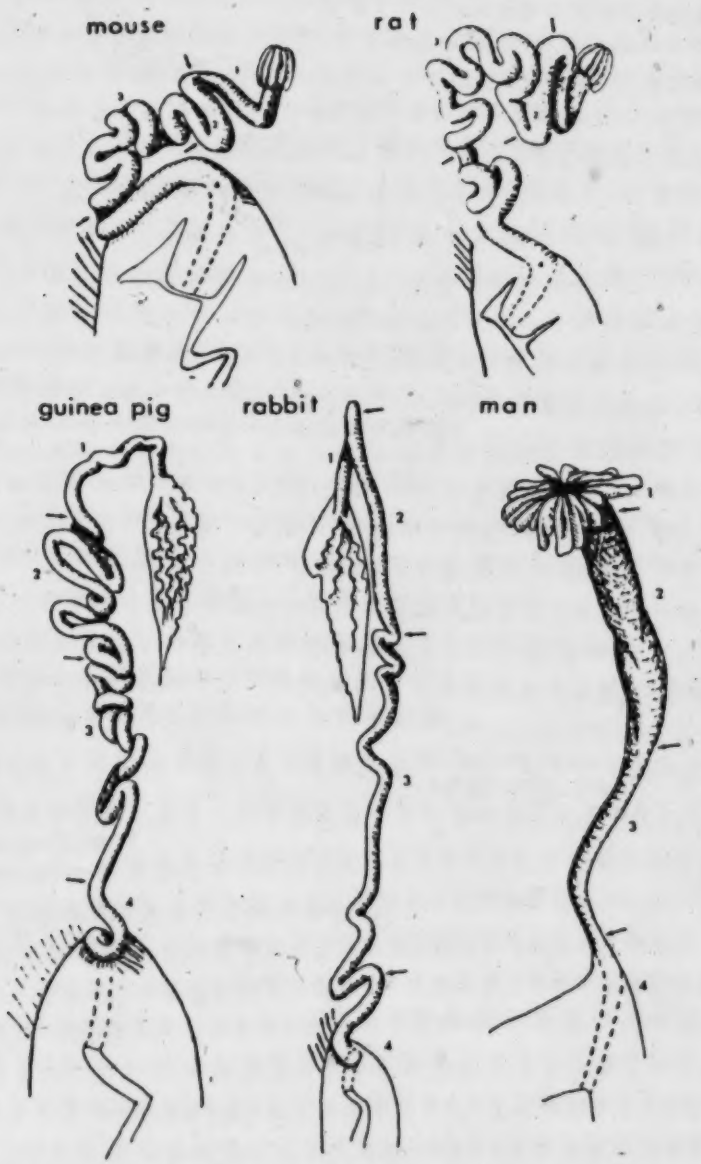

FIG. 1 Diagrammatic representation of the oviducts of the mouse, rat, guinea pig, rabbit and human. The four anatomic segments are demarcated 1. pre-ampulla, 2. ampulla, 3. isthmus, and 4. junctura. These drawings are not to scale (from Nilsson and Reinius, 1969, p. 60).

thickness and arrangement of oviduct smooth muscle may well contribute to variations in the pattern of contraction between ampulla, isthmus and infundibulum and may partially account for inter-species differences in oviduct motility.

The tunica mucosa forms the framework of the mucosal folds into the oviductal lumen. The complexity of these projections varies among species and along the length of the oviduct (usually decreases from the ovarian to uterine end). The patterns of mucosal folding from different segments of the rabbit oviduct are illustrated in Figure 2.

The lamina propria and the lamina epithelialis have been distinguished as two different layers in the tunica mucosa. The lamina propria is a highly vascular, loose connective tissue layer between the muscle and epithelial layers. Cell types in this tissue include fibroblasts, mast cells, blood elements and 'indifferent' cells (Woodruff and Pauerstein, 1969).

The lamina epithelialis is composed of four different cell types; ciliated, secretory, basal and peg cells. Ciliated and secretory cells occur in approximately equal numbers, the ciliated being more numerous at the ovarian end while secretory cell numbers increase towards the uterine end (Patek et al., 1972). Halbert et al. (1976a) have described an important role for cilia in ovum transport through the rabbit oviduct ampulla. A role in ovum transport has also been proposed for tubal secretions (Koester, 1970). These epithelial cells exhibit marked cyclic changes in their height and relative proportions (Patek et al., 1972). Basal cells make up less than one per cent of the total epithelial cells and are irregularly distributed at the base of the epithelium along the length of the tube. Woodruff and Pauerstein (1967) have suggested that these serve as reserve cells which may differentiate into ciliary or secretory cells.

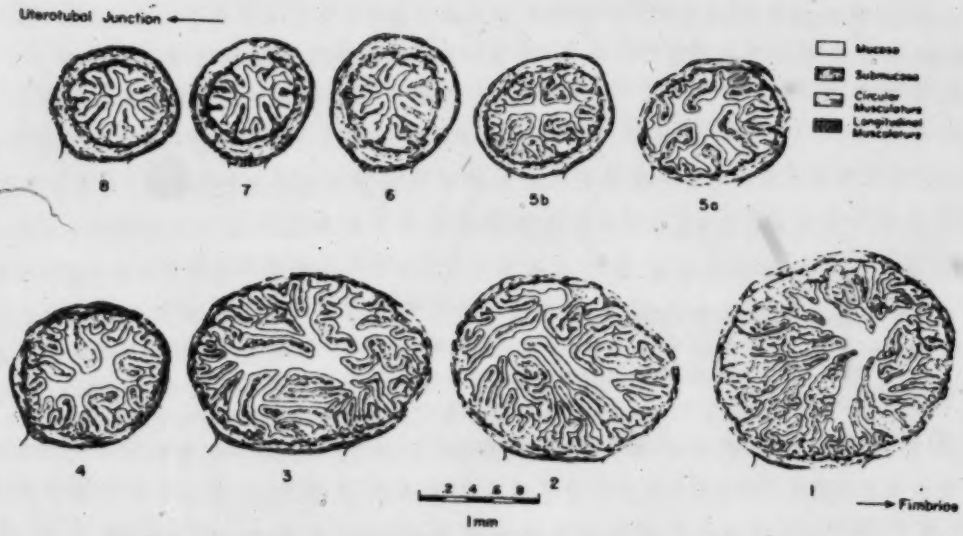

FIG. 2 Diagrammatic illustration of transverse sections of different segments of rabbit oviduct. The figures represent sections from the fimbriae (1), ampulla (2-4), ampullary-isthmic junction (5a, b), isthmus (6,7), and junctura (8) (from El-Banna and Hafez, 1970, p. 475).

Peg cells occur more frequently towards the ovarian end, yet make up only 0.5 - 1% of the total cell numbers. While it has been claimed that the peg cell is an exhausted secretory cell (Novak and Everett, 1928), more recent electron microscopic studies have revealed no transition between these two cell types (Nilsson and Reinius, 1969). These cells also show cyclic variations.

III Embryology

Embryologically, the oviducts and the uterus are derived from the Müllerian (paramesonephric) ducts. The cranial segments of the embryonic ducts differentiate into the paired Fallopian tubes while the caudal part forms the uterine horns.

First evident as an invagination of the coelemic epithelium in the 4-5 week embryo (Woodruff and Pauerstein, 1969), the human oviducts undergo marked growth activity until about 5 months of embryonic life. At birth, the connective, muscular and mucosal layers can be discerned and the pre-ampulla, ampulla, isthmus and junctura are distinguishable. In the rabbit and guinea pig, the oviducts are similarly well developed before birth, but the hamster, rat, and mouse undergo substantial differentiation post-natally, including oviduct demarcation from the uterus (Price et al., 1969).

IV Extrinsic Innervation

A. Cholinergic

The cholinergic innervation to the Fallopian tube arises from two sources. The distal portion of the oviduct is supplied by vagal fibres via the ovarian plexus while the isthmic and junctural portions are served by short postganglionic fibres from the pelvic nerve via the pelvic plexus. These pelvic fibres originate from S_2, S_3 and S_4 sacral nerves (Crosby et al., 1962).

B. Adrenergic

Some preganglionic sympathetic fibres arising from T_{10-12}, $L_{1,2}$ synapse in the inferior mesenteric ganglion from which postganglionic fibres innervate the oviductal isthmus via the hypogastric nerve. Fibres from $T_{10,11}$ also supply the infundibulum and distal ampulla via the ovarian plexus.

The sympathetic innervation to the reproductive tract is remarkable in that it is further comprised of 'short' adrenergic fibres which arise from peripheral uterovaginal ganglia (Owman et al., 1966). These 'short' adrenergic neurons differ from the classical 'long' neurons in certain functional aspects (Sjöberg, 1967; Owman and Sjöberg, 1967) including their susceptibility to the influence of sex hormones (Owman and Sjöberg, 1973; Rosengren and Sjöberg, 1968; Sjöberg, 1968a).

Figure 3 illustrates the extrinsic cholinergic and adrenergic innervation to the mammalian oviduct.

V Intrinsic Innervation

A. Cholinergic

A small number of studies have attempted to demonstrate the intrinsic cholinergic innervation of the mammalian oviduct. These investigations were based on histochemical techniques for the staining of acetylcholinesterase and relied on the assumption that this enzyme reflects the presence of acetylcholine.

In rabbit oviduct, acetylcholinesterase with a distinct localization to nerves was not seen in the ampulla and was evident to only a very slight extent in the isthmus (Owman and Sjöberg, 1966). Jordan (1970) found no evidence of cholinergic innervation to the myosalpinx of guinea pig and rabbit. Rather, acetylcholinesterase activity has been largely localized

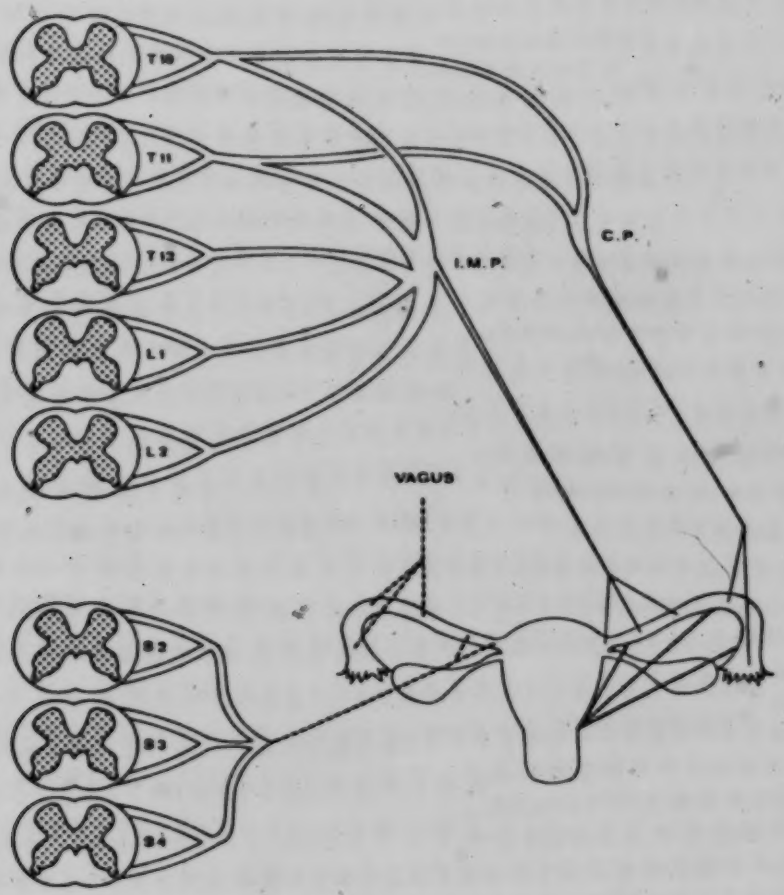

FIG. 3 Diagrammatic representation of the extrinsic innervation of the mammalian oviduct. Sympathetic innervation (solid lines) arises from T_{10-12}, $L_{1,2}$ and also from peripheral uterovaginal ganglia. Cholinergic innervation (broken lines) is supplied by the vagus nerve and also by fibres from S_{2-4} via the pelvic nerve. IMP, inferior mesenteric plexus; CP, coeliac plexus (modified from Woodruff and Pauerstein, 1969, p. 36).

in the mucosal layer (cat, Jacobowitz and Koelle, 1965; human, Kubo et al., 1970). Hence, it is generally considered that the cholinergic innervation to the mammalian oviduct is limited to the mucosa.

B. Adrenergic

In contrast to studies of intrinsic cholinergic innervation, a large number of investigations have employed specific histochemical techniques (Falck et al., 1962) for the localization of catecholamines in adrenergic nerve terminals in the mammalian oviduct.

A common pattern for the distribution of adrenergic nerves has been described in the human (Brundin and Wirsen, 1964a; Owman et al., 1967; Kubo et al., 1970), rabbit (Brundin and Wirsen, 1964b; Owman and Sjöberg, 1966; Brundin, 1965), rat (Brundin et al., 1969), cat (Rosengren and Sjöberg, 1967; Jacobowitz and Koelle, 1965) and dog oviduct (Owman and Sjöberg, 1972). Only a very few adrenergic fibres are present in the fimbria and ampulla and these are almost exclusively associated with blood vessels. In contrast, the isthmus receives abundant adrenergic innervation which is largely localized to the circular muscle layer.

This dense innervation to the isthmus is noteworthy and may well be of functional significance. Norberg (1964) has demonstrated that the smooth muscle of the intestine and ureters are essentially devoid of direct adrenergic innervation except for sphincteric regions. It has been proposed that the oviductal isthmus acts as a sphincter under adrenergic control in the regulation of ovum transport (Brundin, 1965). This will be discussed in a subsequent section.

VI Biochemical Studies of the Sympathetic Innervation of the Oviduct

A. Changes in Noradrenaline Content

1. Measurement of noradrenaline content

The availability of biochemical methods for the quantitative estimation of noradrenaline in biological tissues has made it possible to study changes in oviductal noradrenaline content that may be of physiological importance. Chemical determinations have revealed that noradrenaline is the predominant monoamine in the oviduct (Rosengren and Sjöberg, 1967; Bodkhe and Harper, 1973; Dujovne et al., 1976; Owman et al., 1976) and fluorescence histochemistry has illustrated that the transmitter is stored within neurons (Owman et al., 1966, 1967; Owman and Sjöberg, 1966; Rosengren and Sjöberg, 1967). The noradrenaline content of any given tissue is generally considered to be proportional to the degree of sympathetic innervation; findings in the female reproductive organs of the human (Brundin and Wirsen, 1964a; Owman et al., 1967), rabbit (Brundin, 1965; Sjöberg, 1967), cat (Rosengren and Sjöberg, 1967) and sheep (Holst et al., 1970) are in general agreement with this belief.

Von Euler (1956) has proposed that the activity of adrenergic neurons varies directly with their content of noradrenaline. If this is indeed so, a change in noradrenaline content that is associated with a particular state or function of the oviduct might indicate a functional involvement of the adrenergic nerves in the process. Numerous studies have investigated such a possibility by measuring oviductal noradrenaline content during normal ovum transport (Bodkhe and Harper, 1972, 1973), and by attempting to correlate changes in transmitter content with changes in levels of endogenous sex hormone (Dujovne et al., 1976), with administration of sex hormones (Sjöberg, 1968a; Bodkhe and Harper, 1973; Owman and Sjöberg, 1975;

Moawad and Zuspan, 1975 (abstr.)), with pregnancy (Rosengren and Sjöberg, 1968), or following surgical denervation, 6-hydroxydopamine, reserpine or iproniazid (Brundin, 1965; Owman et al., 1966; Bodkhe and Harper, 1972; Pauerstein et al., 1974c).

A number of methods are available for the estimation of catecholamines in tissues. However, most of the studies of noradrenaline content in the oviduct are based on the trihydroxyindole method (Ehrlen, 1948). While the techniques available for the measurement of noradrenaline have sufficient precision and sensitivity, there is a general inadequacy in the expression of results. This makes it difficult to compare data from different laboratories. For example, the sensitivities of the techniques employed may differ between labs, not all workers report the recoveries they obtained and the oviduct portions studied have frequently differed from study to study.

2. Normal levels

Biochemical analyses of the oviducts of different species have generally shown that noradrenaline levels are higher in the isthmic portion than in the ampullary part. The distribution of noradrenaline in the rabbit oviduct was first examined by Brundin (1965) who reported that the uterine half contained 2.3 ± 1.2 µg/g wet tissue while the ovarian half contained 0.3 ± 0.4 µg/g. Owman et al. (1966) performed their determinations on the whole rabbit oviduct and obtained a value of 0.9 ± 0.1 µg/g wet tissue for the noradrenaline concentration. Bodkhe and Harper (1972) reported noradrenaline levels of 0.9 µg/g for ampulla, 2.2 ± 0.2 µg/g for distal isthmus and 0.5 ± 0.1 µg/g for proximal isthmus of rabbit oviduct. Hence, in these rabbit studies alone, at least three different ways of sectioning the oviduct were used. Thus, one is unable to compare levels in a given portion

of the oviduct, e.g., in the more densely innervated distal isthmus where adrenergic nerves may be of the most physiological significance.

Brundin and Wirsen (1964a) reported approximately equal values (0.1 ± 0.3 µg/g) for the levels of noradrenaline in the isthmic, ampullary and infundibular thirds of the human oviduct. However, Owman et al. (1967) later reported a significantly higher concentration of noradrenaline in the human isthmus (0.5 ± 0.1 µg/g) than in the ampulla (0.3 ± 0.1 µg/g) or intramural portion (0.3 ± 0.03 µg/g). The noradrenaline content was not found to differ, however, between three equal sections of isthmus. These estimations were made on samples from menstruant, non-pregnant females. However, the hormonal status was not defined by either group of investigators. Brundin and Wirsen suggested that the similarity of noradrenaline content in the different oviductal parts, compared to the histochemical findings, could possibly be due to the presence of a plexus of adrenergic innervation along the ampulla and infundibulum which was absent in the isthmic region.

While noradrenaline levels in the human oviduct were found to be substantially lower than those in the rabbit oviduct, the contents of noradrenaline in the cat (Rosengren and Sjöberg, 1967) and sheep (Holst et al., 1970) oviduct have been shown to be comparable to those in the rabbit. Black (1974) has listed in tabular form the noradrenaline contents (in µg/g wet tissue) and the assay method employed for four different species.

There is considerable debate in the literature concerning the advisibility of expressing noradrenaline levels in terms of 'concentration', i.e., µg/g wet tissue. Brundin (1965) has argued rather that the 'content' of noradrenaline, i.e., µg/pair of oviducts, is a more valid measure. Since the weights of oviducts vary even among controls, one can readily

appreciate the increased complications when sex hormones induce water retention, muscle hypertrophy and stimulate secretion, all of which may influence tissue weight and consequently noradrenaline values when expresed as µg/g wet weight. The increase in tissue weight with hormone treatment probably reflects mainly an increase in non-nervous tissue and, therefore, the content of noradrenaline per oviduct may be a more meaningful method of expression (Bodkhe and Harper, 1973). Indeed, many workers have calculated both noradrenaline concentration and content in their assessment of adrenergic influences in oviduct function.

3. Changes in noradrenaline content during normal ovum transport

Studies attempting to correlate levels of noradrenaline with the course of normal ovum transport were performed by Bodkhe and Harper (1972). Rabbits were killed at various times following induction of ovulation by HCG and artificial insemination. The content of noradrenaline was not altered in the proximal isthmus while in the ampulla levels of noradrenaline were significantly less 72 hrs after insemination than in estrus and in the distal isthmus at 17, 50, 72 and 90 hrs than in estrus. The concentration of noradrenaline (µg/g) varied only in the distal isthmus, being significantly less than levels in estrus at 17 hr postinsemination. The most significant finding was the decreased content and concentration of noradrenaline in the distal isthmus at 17 hr since this appeared to be correlated with the passage of eggs from the ampulla into that segment of the isthmus.

However, while correlations may be fairly readily discovered, conclusions concerning the influence of noradrenaline levels on ovum transport are less readily drawn. The relation of the amount of transmitter to physiological function is modulated by many factors, including release,

reuptake and other inactivation processes, receptor sensitivity and so on. Weiner (1970) has warned of another fundamental problem in attempting to correlate noradrenaline content with physiological phenomena: there is more than one noradrenaline compartment or pool in adrenergic nerve endings. Of these, a small, active pool may be of most significance physiologically.

A second finding in the study of Bodkhe and Harper was that the noradrenaline content in the rabbit isthmus remained low during ovum transport when circulating estrogens are reported to be low (Eaton and Hilliard, 1971). Hormone assays were not performed, however.

4. Changes in noradrenaline content : Correlation with endogenous hormone levels

A recent investigation (Dujovne et al., 1976) has attempted to correlate the noradrenaline content of the human oviduct with plasma levels of estradiol and progesterone. In the proliferative phase, when plasma levels of estradiol and progesterone were 171 ± 33 pg/ml and 495 ± 133 pg/ml respectively, the noradrenaline concentration in the isthmus was 2-3 times that in either the infundibulum or ampulla. In the secretory phase following ovulation, when plasma levels of estradiol and progesterone were 249 ± 20 pg/ml and 3,027 ± 162 pg/ml respectively, there was no difference in levels of noradrenaline between the three oviduct segments since noradrenaline levels in only the infundibulum and ampulla increased with the rise in plasma progesterone. Dujovne et al. consider that this correlation between noradrenaline and progesterone in the secretory or luteal phase suggests that ovum passage through the human oviduct may be concomitant with an increase in adrenergic transmitter.

An earlier study (Owman et al., 1967), reported no significant variation in the total content of noradrenaline in the human oviduct between

the proliferative and secretory phases. However, these workers failed to consider cyclical changes in the regional distribution of nordrenaline and did not measure plasma steroid levels. A similar, more recent investigation (Owman et al., 1976) determined that the noradrenaline concentration in the proliferative phase was about twice that in the secretory phase in human and Rhesus monkey oviducts.

Thus, reports in the literature have, for the most part, indicated that the oviduct exhibits differences in noradrenaline content concomitant with changes in hormonal dominance. Dujovne et al. have determined that this is a regional phenomenon in the human oviduct.

5. Effect of sex hormone administration on noradrenaline content

Attempts to establish whether endocrine factors are responsible for changes in transmitter content have involved the administration of exogenous sex hormones and the subsequent measurement of noradrenaline levels.

Bodkhe and Harper (1973) employed doses of estrogen (25 or 250 µg/day) or progesterone (2 mg/day) that are known to either retard or accelerate normal ovum transport in the rabbit (Greenwald, 1961). Treatment with estrogen appeared to have little or no effect on the noradrenaline concentration in the ampulla and proximal isthmus, but did result in elevated noradrenaline contents in these regions and this was associated with an increase in tissue weight. However, in the distal isthmus adjacent to the ampulla, estrogen treatment had pronounced effects: noradrenaline concentration and content were increased without consistent increases in tissue weight. A correlation was observed between these increased noradrenaline levels in the distal isthmus and estrogen-induced retention of eggs at the ampullary-isthmic junction. However, the corollary that progesterone-induced accelerated ovum transport would be associated with decreased levels of noradrenaline in the distal isthmus was not observed.

In fact, noradrenaline concentrations in the distal isthmus were elevated with progesterone treatment. The hormonal modification of ovum transport cannot, therefore, be explained simply in terms of changes in noradrenaline levels.

The literature concerning the effect of administered sex hormones on noradrenaline levels is wrought with inconsistencies in methods and in results. This is illustrated by the following examples. Brundin (1965) treated one group of rabbits with 17 β-estradiol (50 µg i.m./day/8 days) and another group with this estrogen treatment plus progesterone (1 mg i.m./day for the last four days). No significant differences were reported in the mean noradrenaline contents per oviductal pair between the hormonal groups nor between these groups and a control group. Significant variations in oviduct weights were, however, observed (E > E + P > control) and, therefore, expression of noradrenaline levels in terms of concentration (µg/g tissue) would have been substantially different.

Sjöberg (1968b) used much lower doses of estrogen in rabbits (0.5 µg/kg s.c./day for 7 or 14 days). However, he reported that both treatment regimes produced significant increases in noradrenaline content per pair of oviducts. This was associated with significant increases in tissue weight after the 14-day treatment so that, had the noradrenaline level been expressed as µg/g tissue, it would not have been different from controls. In a more recent paper, Owman and Sjöberg (1975) have reported further data on oviductal noradrenaline levels and the effect of exogenous hormones. Treatment with 17 β-estradiol (0.5 µg/kg/day for 14 days) caused a two-fold increase in the levels of noradrenaline in the rabbit oviduct. When progesterone (2 mg/kg/day for the last 7 days) was added to the treatment schedule, transmitter levels did not differ from controls. In both this and the preceding study, changes in noradrenaline

content corresponded to changes in the number of adrenergic nerves detected by histofluorescence microscopy. Other workers (Moawad and Zuspan, 1975) have determined the amount of noradrenaline in groups of control, estrogen and estrogen + progesterone-treated rabbits. They found no significant differences in noradrenaline concentration in oviductal tissue (ug/unit weight).

6. Changes in noradrenaline content during pregnancy

It has been reported that the short adrenergic neurons which supply the female genital tract (uterus, oviduct, vagina) exhibit changes in their transmitter content after administration of sex hormones and during pregnancy (Rosengren and Sjöberg, 1968) while such changes were absent in organs innervated by classical long adrenergic neurones, i.e., heart and ovary (Rosengren and Sjöberg, 1968; Owman and Sjöberg, 1973). This responsiveness to hormonal status may indicate an important physiological distinction between short and long adrenergic neurones.

Rosengren and Sjöberg (1968) reported increased noradrenaline content (x2) in the rabbit oviduct 4-12 days after mating compared to non-pregnant values. An increased number of nerves paralleled increases in oviduct weight. These results are similar to those determined by Sjöberg (1968b) and Owman and Sjöberg (1975) following estrogen treatment.

In summary, there is evidence that changes in noradrenaline content may occur with changes in hormonal status. The phenomenon appears to be specific to the reproductive tract but not to the ovary (Owman and Sjöberg, 1973; Sjöberg, 1967) and hence may be related to the unique innervation of these tissues by short adrenergic neurons.

7. Effect of agents which affect noradrenaline levels

Surgical denervation techniques have been used to define the origin of the innervation to the female reproductive tract. These studies have

usually involved sectioning of the hypogastric nerve at the level of the inferior mesenteric ganglion. Owman et al. (1966) reported a 50% reduction in the noradrenaline content and concentration in the rabbit oviduct following denervation. Brundin (1965) found that the mean content of noradrenaline was reduced to one-third of that in oviducts of sham-operated or control rabbits. Hence 30-50% of the total innervation of the rabbit oviduct probably consists of short adrenergic neurones. Such depletion following denervation was not seen in the cat (Rosengren and Sjöberg, 1967). Owman et al. (1966) have reported complete abolition of fluorescence following sectioning of the hypogastric nerve at the levels of the inferior mesenteric ganglion and utero-vaginal levels combined with stripping of the vaginal fascia.

An alternate procedure to produce catecholamine depletion is the use of reserpine. Brundin (1965) found that reserpine (1 mg/kg for 2 days or 0.25 mg/kg 20 hr before sacrifice) depleted noradrenaline below measurable levels. Bodkhe and Harper (1972) injected rabbits with reserpine (0.25 mg/day) on the day before HCG/insemination and until autopsy at 26, 50 and 90 hr after HCG. There was a significant reduction in noradrenaline levels (content and concentration) in the isthmus; the most pronounced depletion was noticed in the distal isthmus since it contained the highest noradrenaline concentrations normally. Although the ampulla was depleted of noradrenaline to levels less than 10 ng, these amounts were not significantly different from controls.

A third method for causing depletion of noradrenaline from sympathetically innervated organs is the use of 6-hydroxydopamine. Administration of this agent causes the selective destruction of peripheral adrenergic nerve terminals in adult animals. Black (1974) measured changes in noradrenaline content following perfusion of the rabbit oviduct with

6-hydroxydopamine. The range of 6-hydroxydopamine doses used (0-20 mg) induced decreased levels of noradrenaline, and the segments closest to the uterus were most sensitive to this effect. At high doses, however, the contralateral, control oviduct was also affected. Eddy and Black (1973) have discussed the need for controlled chemical denervation of individual oviducts. They suggested that 1-5 mg 6-hydroxydopamine was optimal to cause denervation but insufficient to produce significant systemic effects. Chemical sympathectomy of the rabbit oviduct using 6-hydroxydopamine perfusion has been studied in relation to ovum transport and will be discussed in a later section.

Iproniazid, a monoamine oxidase inhibitor, has been utilized to raise oviductal noradrenaline levels (Bodkhe and Harper, 1972). The influence of iproniazid on ovum transport will be discussed later. The working hypothesis was that if an increased noradrenaline content alone was associated with altered oviduct function, one could surmise that noradrenaline levels might mediate hormonal influences on the oviduct.

The preceding discussion has dealt with changes in noradrenaline content in the oviduct during normal ovum transport, correlated with endogenous sex hormone levels or following the administration of sex hormones, during pregnancy and subsequent to treatment with chemicals which alter noradrenaline levels. A fundamental problem exists when one measures content changes since it is not possible to determine whether the change is due to altered synthesis and storage of noradrenaline or to changes in the metabolism of the transmitter.

B. Biosynthesis of Noradrenaline

It is presumed that, under steady-state conditions, estimates of the turnover of noradrenaline yield information regarding the rate of synthesis

(Costa and Neff, 1966), and that this rate of synthesis is related to impulse activity in the sympathetic nerves (Roth et al., 1967; Axelrod, 1972; Gordon et al., 1966). Several authors have submitted that estimation of the rate of catecholamine turnover provides more information about neuronal activity than measurements of amine concentration in the tissue (see for example, Oliverio and Stjärne, 1965; Doteuchi and Costa, 1973). However, measurement of turnover rates may suffer from a similar drawback that affects measurement of content: the turnover of a larger, more stable pool of noradrenaline may be reflected in turnover estimates while the turnover of a smaller pool may be much more rapid and of more significance physiologically (Weiner, 1970).

Takeda and Doteuchi (1976) estimated rates of noradrenaline turnover in the rabbit oviduct under different hormonal conditions. The initial rate of decline of noradrenaline following blockade of its synthesis with α-methyl-p-tyrosine methylester was used as an estimate of noradrenaline turnover rate. The turnover rate was slightly higher in estrogen-dominant tissues (0.41 nmoles/g/h) than in progesterone-dominant tissues (0.30 nmoles/g/h). No such differences were found in atria which is innervated by 'long' adrenergic neurons. Hence, the intermediate effect of hormones on noradrenaline turnover in the oviduct could be explained by its mixed ('long' and 'short') innervation.

Recently, Kennedy and Marshall (1977) have performed similar estimations of noradrenaline turnover rates in rabbit oviduct. While the rate constant for the decline of tissue noradrenaline was significantly lower in castrates than hormone-treated oviducts, they found no difference in turnover rates between estrogen- and progesterone-dominant tissues. This is in disagreement with the observation by Takeda and Doteuchi (1976) of a higher turnover rate in estrogen-treated rabbits. However, Takeda

and Doteuchi administered 10-fold greater doses of estrogen. This work requires verification, therefore, and would also benefit from separate analysis of turnover rates in the isthmus, ampulla and infundibulum.

C. Metabolism and Uptake of Noradrenaline

The physiological disposition of monoamines is another factor which may contribute to changes in noradrenaline content or turnover. Hormones have been shown to modify the metabolism of labelled amines in rat uterus (Parvez et al., 1976; Collins and Southgate, 1970) but similar studies have not been performed in the oviduct. It would be of considerable in interest to measure the activities of monoamine oxidase and of catechol-O-methyl transferase in the oviduct following treatment with estrogen or progesterone.

Neuronal uptake is considered to be the major route of inactivation of noradrenaline in oviduct isthmus (Johns and Paton, 1975; 1976). However, few studies have investigated directly neuronal uptake processes in this tissue. Paton and Johns (1975a) have characterized the accumulation of [^{3}H](±)-metaraminol, a sympathomimetic amine, in human oviduct. The accumulation of metaraminol was decreased by inhibitors of neuronal uptake but not by extraneuronal uptake blockers. The accumulation of labelled metaraminol was greater in isthmic tissues than ampulla: this reflects the more dense innervation of the isthmic smooth muscle. Such a measurement of neuronal uptake would be a valuable tool in the investigation of possible hormonal modifications of the uptake process in the oviduct.

D. Summary

In summary, it may be said that while techniques are available for the measurement of noradrenaline content, turnover and metabolism, there are problems and omissions in their application to the study of oviduct

function. Important difficulties remain in establishing cause-and-effect type relationships between noradrenaline levels and turnover of transmitter and oviduct physiology. A more complete analysis would have to include investigation of noradrenaline release and effector adrenoceptor sensitivity under the various experimental conditions.

VII Adrenergic Receptors in the Mammalian Oviduct

A. Introduction

The first pharmacological studies performed on the oviduct revealed that the smooth muscle of the human and pig oviduct was sensitive to adrenergic stimulation (Kok, 1926, 1927). Davids and Bender (1940) measured *in vivo* motility of rabbit oviduct by the Rubin tubal insufflation technique to monitor contractile responses to adrenaline. It is interesting that these early workers reported the importance of hormonal status on the sensitivity and nature of the response of the oviduct to adrenergic drugs.

Subsequent to Ahlquist's study (1948) of the adrenotropic receptors, investigators employed the selective application of adrenergic agonists and antagonists to define the adrenoceptors in a large number of smooth muscle preparations including the oviduct. Evidence has accumulated to indicate the presence of both α-excitatory and β-inhibitory adrenoceptors in the oviduct of various mammalian species.

B. Rabbit Oviduct

In the rabbit oviduct, adrenaline, noradrenaline and phenylephrine elicit contractile responses which are antagonized by the α-adrenoceptor blocking agents phentolamine or phenoxybenzamine (Longley *et al.*, 1968; Coutinho *et al.*, 1971; Levy and Linder, 1972; Howe and Black, 1973; Ueda *et al.*, 1973; Paton *et al.*, 1975; Kendle and Lam Shang Leen, 1976).

Stimulation of rabbit oviduct *in vivo*, by hypogastric nerve stimulation (Brundin, 1965) and *in vitro*, by transmural stimulation (Hodgson *et al.*, 1973; Johns and Paton, 1975; Paton *et al.*, 1975) also causes contractile responses which are eliminated by α-adrenoceptor blockade. The contractile effects of hypogastric nerve stimulation are also blocked by pretreatment with reserpine (Brundin, 1965) while the contractile effects of transmural stimulation are blocked by tetrodotoxin, guanethidine and by pretreatment with 6-hydroxydopamine (Hodgson *et al.*, 1973; Johns and Paton, 1975). These results indicate that these contractile responses are caused by the activation of α-adrenoceptors by neurotransmitter released from adrenergic nerve endings.

The β-agonist, isoprenaline, inhibits tubal motility in the rabbit and this is inhibited by propranolol (Longley *et al.*, 1968; Martin *et al.*, 1970; Coutinho *et al.*, 1971; Levy and Linder, 1972; Howe and Black, 1973; Hodgson and Pauerstein, 1974; Heilman *et al.*, 1972; Spilman and Harper, 1974; Heilman *et al.*, 1976; Kendle and Lam Shang Leen, 1976). In the presence of phenoxybenzamine, the response to noradrenaline (formerly contraction) was reversed and was expressed as a relaxation (Ueda *et al.*, 1973). The occurrence of β-receptors in the rabbit oviduct was thus confirmed in these studies, and activation of β-adrenoceptors mediates relaxation in the rabbit oviduct.

Recently, Kendle and Lam Shang Leen (1976) have endeavored to further define the type of β-adrenoceptor in the rabbit oviduct. They reported that, in the presence of practolol, the contractile response to adrenaline was potentiated in circular muscle but not in the longitudinal layer. Salbutamol, a β_2 agonist, was found to cause inhibition in the longitudinal muscle layer but not in the circular layer. From these results they suggested that the inhibitory receptor in the longitudinal muscle is of the

β_2 type, while that in the circular muscle is of the β_1 type. No data were presented, however. Their proposal may be severely criticized when extended to explain the difference in relative potency of adrenaline (α, β_1, β_2) and noradrenaline (α, β_1) in the two smooth muscle layers since routes of agonist inactivation were not considered in their study. It has been shown that noradrenaline is a better substrate for neuronal uptake than adrenaline (Iversen, 1967) and that, since circular muscle is more densely innervated, the influence of neuronal uptake will be significantly greater in circular than in longitudinal muscle. In fact, the difference in sensitivity to noradrenaline between circular and longitudinal muscle layers can be largely eliminated by cocaine, desipramine or 6-hydroxydopamine which prevent the neuronal uptake of amines (Johns and Paton, 1975, 1976).

There has been at least one biochemical study of adrenergic receptors in the rabbit oviduct (Chatkoff and Pauerstein, 1975 (abstr)). Catecholamine binding sites were shown to be predominantly located in the microsomal fraction by ^{3}H-noradrenaline binding assays. Mean levels of receptor sites were estimated at 4.5 pmole/unit microsomal fraction and the density of receptor sites in the oviductal segments was shown to vary by more than 400% from the ampulla to the utero-tubal junction.

However, the interpretation of such receptor binding assays using labelled catecholamines is difficult because: 1) the binding is not stereospecific, 2) blocking agents have little or no effect on the degree of binding, and 3) there is a discrepancy between the time course of catecholamine binding and the more rapid stimulation of biochemical activity, e.g., adenylate cyclase activity (see reviews by Cuatrecasas, 1974; Bär, 1976). Indeed, labelled agonist binding is generally considered to reflect a non-specific, irreversible, catechol-directed binding component

which does not represent interaction with adrenergic receptors.

A growing body of evidence indicates that the use of labelled antagonists ([^{3}H](±)-propranolol, [^{3}H](-)-alprenolol, [^{125}I]-hydroxybenzylpindolol) provides a more reliable identification of β-adrenoceptors (Levitzki et al., 1974; Lefkowitz et al., 1974; Mukherjee et al., 1975; Aurbach et al., 1974). The antagonists exhibit a higher apparent affinity for the putative receptor site than agonists and lack the catechol structure associated with non-specific binding.

C. Human Oviduct

Similarly, α-excitatory and β-inhibitory responses which may be blocked with suitable adrenergic blocking agents have been demonstrated in the human oviduct. In 1960, Sandberg et al. measured contractions of longitudinal muscle following administration of adrenaline or noradrenaline to the preparation. Another in vitro investigation of autonomic responses of human Fallopian tube (Rosenblum and Stein, 1966) monitored changes in perfusion pressure following challenge with noradrenaline and isoprenaline. Both caused an increase in perfusion pressure which was attributed to contraction of the circular muscle layer. In the presence of phenoxybenzamine, this response was reversed to one of relaxation and hence, indicated the presence of both α- and β-receptors.

Nakanishi and Wood (1968) administered various β-receptor stimulants (isoprenaline, adrenaline in the presence of phenoxybenzamine) to human oviduct in vitro and recorded decreased muscular tone.

However, results from various laboratories are not in complete agreement with those just presented. In vitro fluid perfusion studies of human isthmus (Seitchik et al., 1968) reported that, in response to noradrenaline, one-third showed no change and one-third exhibited a decrease

in perfusion pressure. However, in the presence of β-blockade, an increase in circular muscle tone was the predominant response. Other studies (in vitro, Senior and Spencer-Gregson, 1969; in vivo, Cibils et al., 1971) reported biphasic responses of human oviduct to noradrenaline - a contraction (blocked by phentolamine) followed by a predominant relaxation (reversed by propranolol).

Studies of the responses of isolated oviduct to perivascular nerve stimulation (Nakanishi et al., 1967; Nakanishi and Wood, 1968) and following transmural stimulation (Paton et al., 1975; Molnar et al., 1976) have, in similar fashion, produced conflicting results. While α-excitatory and β-inhibitory receptor activity could be demonstrated in each study, the predominant response to adrenergic nerve stimulation varied.

D. Other Species

The effects of catecholamines during anestrus and estrus were investigated in rat oviduct in the presence or absence of α- and β-blockers (Borda et al., 1975). The inhibition of motility elicited during anestrus was abolished by propranolol, and hence was attributed to β-receptor activity. During estrus, phenylephrine (in the presence of propranolol) stimulated the rat oviduct to contract. Thus, α- and β-receptors were demonstrated with special consideration for hormonal status.

Ruckebusch and Pichot (1975) studied the effects of adrenergic drugs on sheep oviduct motility in vivo. While phenylephrine and noradrenaline (α-agonists) induced contractile responses, and isoprenaline (β-agonist) caused relaxation, selective blocking agents were not employed to further confirm the presence of both α- and β-receptors. α-Excitatory and β-inhibitory responses have also been demonstrated in guinea-pig oviduct (Gimeno et al., 1976).

In summary, then, α- and β-adrenoceptors are present in the oviducts of all species studied. Activation of α-adrenoceptors results in contraction of oviductal smooth muscle while activation of β-adrenoceptors results in inhibition of contractility.

E. Hormones and Adrenoceptor Sensitivity

Early experiments revealed that estrogen and progesterone were involved in the regulation of the cyclic nature of muscular activity in the female reproductive tract (Robson, 1937; Gennell, 1940). The general finding was that estrogen activates and progesterone inhibits the physiological and pharmacological sensitivity of the smooth muscle of the oviduct. For example, administration of estrogen to castrate rabbits induces an increase in the frequency of tubal contractions (Coutinho and de Mattos, 1968) while progesterone reduces both the frequency and the amplitude (Coutinho, 1973). Similarly, the tubal response to noradrenaline is depressed during progesterone dominance (Higgs and Moawad, 1974; Coutinho et al., 1970).

In ovariectomized rabbits the uterus becomes quiescent almost immediately while the oviduct retains its motility for several weeks following the ablation of the sex hormone source (Coutinho et al., 1971). Thus, tubal motility is under some degree of local control by adrenergic nerves; this is in contrast to the complete systemic control of the rabbit uterus. Many investigations have attempted to elucidate the interplay between the ovarian hormones and oviductal adrenergic mechanisms. This might involve changes in the tissue neurotransmitter content, changes in the relative number and/or sensitivity of tissue receptors, differences in the affinity between adrenergic agonist and receptors, or variations in transmitter release or inactivation mechanisms.

The importance of hormonal status has been mentioned with respect to the catecholamine content of 'short' adrenergic neurons and has been alluded to with respect to the sensitivity and nature of the oviduct tissue response to adrenergic drugs. Ruckebush and Pichot (1975) have detected increased reactivity of the sheep oviduct at the onset of and during estrus. However, Holst et al. (1970) have demonstrated that the noradrenaline content of the sheep Fallopian tube does not change during this period. Rather, the subject which has attracted the most interest is the importance of hormonal status on receptor activation and oviduct motility.

At least two research teams have attributed the effect of progesterone to a decrease in α-adrenoceptor sensitivity. Hunter and Kendle (1974) came to this conclusion upon finding that the response of isolated oviducts from progesterone-dominant rabbits to adrenaline was less than from estrogen-dominant rabbits. However, they cannot fairly claim to have monitored pure α-activity since they used adrenaline, a mixed agonist with α and β activity, in the absence of a β-blocker. The conclusion of Heilman et al. (1976) which attributed decreased sensitivity of rabbit oviduct to noradrenaline during early gestation (progesterone dominant) to decreased α-sensitivity can be criticized for the same weakness.

Several workers have suggested that progesterone accomplishes its inhibitory influence through increased β-receptor activity. In 1970, Martin et al. demonstrated that the β-inhibitory response to isoprenaline was consistently elicited in a progesterone group but was variable in an estrous group. Coutinho et al. (1971) claimed that enhancement of β-stimulation was indicated by the finding of an intensified response of rabbit oviduct to isoprenaline in a progesterone group. Isoprenaline has also been shown to cause greater inhibition of acetylcholine-induced contractions

in progesterone-treated tissues than in estrogen-treated (Hodgson and Pauerstein, 1974, 1975).

Similarly, there are proponents both of increased α-adrenoceptor sensitivity (Howe and Black, 1973) and of reduced β-responsiveness (Black, et al., 1976) with estrogen treatment.

Changes in the relative sensitivity of the two adrenergic receptors with hormonal dominance may be of physiological significance; for example, this may control oviduct motility related to ovum transport.

VIII Ovum Transport

A. Normal Ovum Transport

The transport of ova from the ovarian follicles to the site of implantation is a fundamental step in the reproductive process in the female and is a major function of the oviduct or Fallopian tube. A highly regulated transport is necessary for fertilization, maturation and successful implantation of the egg (Chang, 1950).

Normally, rabbit ova pass within minutes through the ampullary portion (Harper, 1966; Boling and Blandau, 1971), pause for several hours at the ampullary-isthmic junction (Greenwald, 1961) and then move slowly through the isthmus to enter the uterus some 72 hours after the ovulatory stimulus (Pauerstein et al., 1974a). The time course for rabbit ovum transport is illustrated in Figure 4.

A number of studies have investigated tubal motility or contractility, especially with reference to the transient retention of ova at the ampullary-isthmic junction. Brundin (1965) has suggested that ovum transport through the rabbit oviduct is controlled by the sympathetic innervation of the isthmic portion.

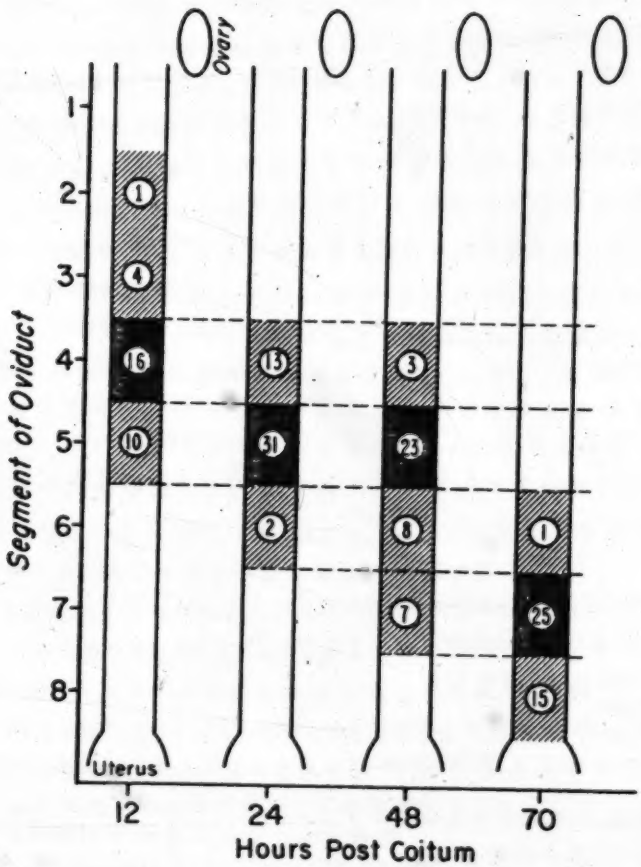

FIG. 4 Distribution of rabbit ova during the normal 3-day passage through the oviduct. Numbers indicate the total number of ova found within each segment of the oviduct (from Greenwald, 1961, p. 82).

From biochemical, histological and histochemical studies of the rabbit oviduct Brundin suggested that the muscular and neuronal arrangements in the oviductal isthmus were such that the isthmus could possess a constrictive, sphincteric function, regulated by adrenergic nerves. Hypogastric nerve stimulation elicited an occlusive response in isthmus which was not obtained from the ampulla or infundibulum. Decreased sensitivity of the isthmus to nerve stimulation and exogenous noradrenaline was recorded during the time of ovum passage through the isthmus. Brundin proposed that the circular muscle of the isthmus was a functional sphincter under adrenergic control which had an important role in the regulation of ovum transport.

B. Effect of Agents which Affect Levels of Oviductal Noradrenaline on Ovum Transport

The involvement of the adrenergic nervous system in normal ovum transport has been investigated *in vivo* by the administration of drugs or procedures which affect levels of noradrenaline in the oviduct.

Surgical denervation has been considered as a potential tool for studying the relation between oviductal noradrenaline levels and physiological function. However, Marshall (1970) has questioned whether complete denervation of the oviduct can ever be accomplished surgically due to the heterogeneous origin of its innervation. Surgical denervation has been reported to have no effect on tubal ovum transport (Pauerstein *et al.*, 1974c) but the results must be interpreted cautiously.
Lack of specificity for the adrenergic system, erratic depletion, and postoperative adhesions are detriments to the use of surgical denervation in physiological studies (Black, 1974).

Reserpine depletion of noradrenaline from adrenergic nerve endings has also been employed in studies of oviduct function. Some studies report

that ovum transport and fertility are not significantly affected by reserpine (Kendle and Bennett, 1969b); Pauerstein et al., 1974c; Hodgson et al., 1975). On the other hand, a delay in transport has been reported following systemic reserpine treatment in rabbits (Bodkhe and Harper, 1972) and mice (Bennett and Kendle, 1967), although the effect in mice has been attributed to a centrally-induced hypothermia (Kendle and Bennett, 1967a). The use of reserpine in such functional studies has further limitations since it also may cause interference with gonadotropin release and inhibition of ovulation (Chatterjee and Harper, 1970).

Chemical sympathectomy of the oviduct with 6-hydroxydopamine has also been reported to have no effect on ovum transport. While Castrén et al., (1973) demonstrated that pretreatment with 6-hydroxydopamine prevented pregnancy in rabbits, it is not clear whether this was a consequence of altered ovulation, transport or implantation processes. There are, however, several reports that chemical sympathectomy does not alter rabbit ovum transport or fertility (Eddy and Black, 1974; Pauerstein et al., 1974c). Similarly, 6-hydroxydopamine did not modify fertility in mice or rats (Johns et al., 1975; MacDonald and Airaksinen, 1974). Thus, ovum transport and implantation apparently can proceed normally despite a reduced adrenergic influence in the oviduct. However, 6-hydroxydopamine-induced sympathectomy is associated with denervation supersensitivity (Trendelenburg, 1972; Johns and Paton, 1974). Hence, supersensitivity of the oviduct to circulating noradrenaline might compensate, at least in part, for the degeneration of the adrenergic supply (Paton, 1976).

Iproniazid, a monoamine oxidase inhibitor which elevates oviductal noradrenaline levels, was found to accelerate ovum transport in rabbits (Bodkhe and Harper, 1972). However, iproniazid also inhibits other enzymes, e.g., microsomal liver enzymes (Everett and Wiegand, 1961) and causes

increased motor activity and sympathomimetic signs in rabbits by the fourth day of treatment (Brodie et al., 1959).

In summary, then, various procedures which deplete adrenergic nerves fail to disrupt normal ovum transport. One cannot exclude the possibility that a residual but sufficient adrenergic reserve, or oviductal muscle hypersensitivity might still have permitted normal transport. Considering the interaction of muscle, cilia, secretion and the autonomic nervous system which probably is involved in normal transport, interference with the adrenergic system might be compensated for by changes in the contribution of some other component (Greenwald, 1976). In spite of these possibilities and the downfalls of the experimental techniques employed, but in view of the experimental results, it appears that sympathetic nerves do not play a critical role in the regulation of normal ovum transport.

C. Effects of Adrenergic Agonists and Antagonists on Ovum Transport

The effects of adrenergic drugs on ovum transport have been studied in the rabbit. Clearly, adrenergic agonists are able to alter tubal motility. However, adrenergic agonists and antagonists have uniformly failed to cause major disruption of ovum transport.

Phenoxybenzamine (i.e., blockade of α-adrenoceptors) produced a marginal inhibition of transport in two studies (Langley et al., 1968; Polidoro et al., 1973), but more recently has been reported to have no effect on fertility in rabbits (Hodgson et al., 1975). Propranolol (i.e., blockade of β-adrenoceptors) had no effect on ovum transport or fertility in rabbits (Polidoro et al., 1973; Hodgson et al., 1975).

Adrenaline (750 µg/kg, 32 hr post coitum) only slightly accelerated the rate of transport and only at 2 of 6 time intervals post coitum (Longley et al., 1968). Phenylephrine, administered in high doses and

at frequent intervals, decreased fertility in rabbits but it was not ascertained whether this was a consequence of retarded transport due to oviduct constriction, of accelerated transport due to increased propulsive forces of the oviductal muscle, or of some other action (Hodgson et al., 1975).

These data further suggest that the sympathetic nervous system plays a minor role in normal ovum transport.

D. Effect of Exogenous Hormones on Ovum Transport

The time course of ovum transport is vulnerable to interference by certain sex hormone treatments which have been shown to induce the acceleration or tubal arrest of ova.

In 1935, Burdick and Pincus observed that, following postcoital treatment with estrogenic substances, ova remained in the rabbit oviduct for several days beyond the normal term. Many subsequent studies have shown this inhibition of rabbit ovum transport by a single large dose (250 µg) of estrogen on the day of ovulation (Greenwald, 1961; Chang, 1966; Chang and Harper, 1966; Pauerstein et al., 1970; Pauerstein et al., 1974a). Greenwald (1967) has described a number of species differences for the effect of exogenous estrogen on ovum transport.

On the other hand, pretreatment with progesterone (2.5 - 5 mg) for 3 days prior to ovulation causes acceleration of ovum transport: a high percentage of ova are present in the uterus on day 2 rather than day 3 after the ovulatory stimulus (Greenwald, 1961; Chang, 1967; Kendle and Telford, 1970; Pauerstein et al., 1974a).

Whereas the involvement of the sympathetic nervous system in normal ovum transport is debatable at best, a role is indicated for the adrenergic system in mediating hormonal modification of ovum transport. Both

the estrogen-induced arrest and progesterone-induced acceleration of rabbit ova are antagonized by 6-hydroxydopamine and surgical denervation (Pauerstein et al., 1974c). Further, the estrogen effects, but not those of progesterone, are inhibited by phenoxybenzamine (Pauerstein et al., 1970) and reserpine (Pauerstein et al., 1974c). Therefore, it is believed that the effects of exogenous estrogen are indeed mediated by the intrinsic adrenergic innervation of the oviduct while a more tentative role for the sympathetic system is considered to be involved in the effects of exogenous progesterone on ovum transport.

IX Statement of the Problem

The transport of ova through the oviduct may involve a number of mechanisms: muscular contractions of the tubal wall; epithelial ciliary activity; and the flow of oviduct secretions. Definitive information about the relative contribution of the forces responsible for ovum transport is not available. However, it is generally believed that ciliary activity is of more importance in the pre-ampulla and ampulla than in the isthmus (Clewe and Mastroianni, 1958; Blandau, 1969; Gaddum-Rosse and Blandau, 1976; Halbert et al., 1976b). There is at least one proponent of the importance of isthmic secretory activity in regulating ovum transport (Koester, 1969). Peak secretion and flow towards the ovary occurs on the first post-ovulatory day. Koester has related the decreased flow on the following days with a decreased resistance to ovum transport and subsequent entry into the isthmus.

However, it is considered that the muscular activity of the ampullary-isthmic junction and isthmus contribute directly to the transient arrest of ova at the ampullary-isthmic junction and subsequent slow movement of ova through the isthmus (Brundin, 1965; Blair and Beck, 1976;

Gomez and Croxatto, 1977; Hodgson et al., 1977). Indeed, most of the work on ovum transport has focused on the muscular activity of the oviductal isthmus.

Histological studies have revealed a fairly rich adrenergic innervation to the circular muscle of the oviductal isthmus. Pharmacological investigations have demonstrated that the isthmic smooth muscle is responsive to sympathomimetic drugs and antagonists. The presence of α- and β-adrenoceptors has been well documented. These facts suggest that there may be a role for the adrenergic nervous system in oviduct function.

The cyclic nature of the motility of the entire female genital tract has been known for some time and it has long been recognized that ovarian hormones play a major role in its regulation. While uterine motility is almost completely controlled by the systemic hormonal influences, the rabbit oviduct is considered to be under some local control by its adrenergic innervation.

Sex steroids can alter the rate of ovum transport and have also been shown to alter the response of oviduct smooth muscle to noradrenaline. Indeed, a role has been postulated for the adrenergic nervous system in mediating hormonal influences on ovum transport. A complete analysis of the relationships between steroid hormones, the adrenergic nervous system and oviduct physiology would investigate changes in tissue neurotransmitter content, the relative number and/or sensitivity of tissue receptors, differences in the affinity between adrenergic agonists and receptors, variations in transmitter release or inactivation processes, and changes in oviduct motility or responsiveness to adrenergic agonists under various hormonal conditions.

In the present study we have investigated the effects of transmural stimulation and of adrenergic agonists on the contractility of isthmic

circular muscle of rabbits in estrus and after treatment with sex hormone schedules known to disrupt normal transport. We studied the effect of blockade of neuronal and extraneuronal uptake and of β-adrenoceptor blockade to define the possible role of these adrenergic mechanisms in mediating hormonal control over oviduct function. We attempted to characterize the α- and β-adrenoceptors in oviductal isthmus by determining the order of potency of adrenergic agonists on each receptor system. We further defined the β-adrenoceptor subtype by calculating pA_2 values for certain competitive β-antagonists.

METHODS

I Animals and Hormone Pretreatment

Mature, female New Zealand White rabbits weighing 3-5 kg were employed in the study. They were housed, one or two per cage, in a constant temperature environment (21°C), with an artificial lighting cycle of 12 hr light and 12 hr dark. The animals were supplied with water and commercial rabbit pellets ad libitum.

Three hormone groups were established. Rabbits in GROUP I: Estrus received daily injections of 17β-estradiol (5 μg, s.c.) for 6 days prior to sacrifice. GROUP II: Human Chorionic Gonadotropin (HCG) + Progesterone-pretreatment received progesterone (2.5 mg, i.m.) on the day of HCG injection (100 iu, i.v.) and on each of the two preceding days. This treatment accelerates ovum transport (Greenwald, 1961; Pauerstein et al., 1974a). GROUP III: HCG + Estrogen received a single dose of depo-estradiol (250 μg, i.m.) immediately following HCG injection. This treatment causes retention of ova in the oviduct (Greenwald, 1961; Pauerstein et al., 1974a). Rabbits in Groups II and III were killed 20 hr after HCG administration.

II Measurement of Contractile Responses

The rabbits were killed by cervical dislocation effected by a blow to the neck. The abdomens were opened and the genital tracts were excised and placed in a modified Krebs solution. Uterine histology was examined to define hormonal status.* After the oviducts had been dissected free of surrounding tissue, the isthmus was identified and cut into 2-3 mm pieces. The oviduct segments were threaded twice through the lumen, providing a small loop for attachment to a platinum hook electrode at the bottom of

* Samples of uterine tissue were fixed in formol saline (10%), dehydrated in alcohol, embedded in paraffin, sectioned, and stained by Johnson's Method for Metachromasia (0.1% toluidine blue) (Luna, 1968, p.162). In the Estrous group the endometrium exhibited a proliferative phase typical of estrogen dominance; HCG + Progesterone-pretreated endometrium exhibited a secretory phase typical of progesterone dominance; HCG + Estrogen tissues revealed a mixed hormonal status.

the tissue bath and a second thread for attachment to a transducer. These circular muscle preparations were set up under 0.5 g resting tension in 5-ml tissue baths containing modified Krebs solution at 28°C, pH 7.4 and bubbled with 95% O_2/5% CO_2 (see Fig. 5). Tissues were allowed to equilibrate for at least 45 min before any experimental procedure was begun.

Tissue responses to transmural stimulation or to adrenergic agonists were measured as changes in isometric tension with Grass (FT.03C) Force Displacement Transducers and displayed on a Grass (Model 5D) Polygraph. Transmural stimulation of the oviduct segment was effected by passing biphasic pulses between the two platinum electrodes using a Grass (Model S6) Stimulator. Adrenergic agonists were added to the bath and tissue responses were allowed to reach a maximum before the drugs were washed out.

III Drugs and Chemicals

The following drugs were used in this study: acetylcholine hydrochloride (Sigma), (-)-adrenaline bitartrate (Sigma), cocaine hydrochloride (British Drug Houses, Ltd.), 17β-estradiol (Calbiochem), estradiol cyclopentyl proprionate (depo-estradiol; ICN, Ltd.), guanethidine monosulphate (Mount Royal Chemicals, Ltd.), human chorionic gonadotropin (Sigma), hydrocortisone 20-sodium succinate (Sigma), (±)-isoprenaline hydrochloride (Sigma), papaverine hydrochloride (Eli Lilly & Co.), phenoxybenzamine hydrochloride (Smith, Kline & French), phentolamine mesylate (CIBA), (-)-phenylephrine hydrochloride (Sigma), practolol acetanilide (Ayerst), pregnen-3,20-dione (progesterone; Sigma), DL-propranolol hydrochloride (Sigma), (±)-salbutamol sulphate (A & H, Ltd.) and tetrodotoxin (Sigma).

Stock solutions of the adrenergic agonists were prepared in 10^{-4}M HCl and were renewed every 6-8 days. These drug solutions were refridgerated when not in use. Dilutions were made with modified Krebs solution on the day of

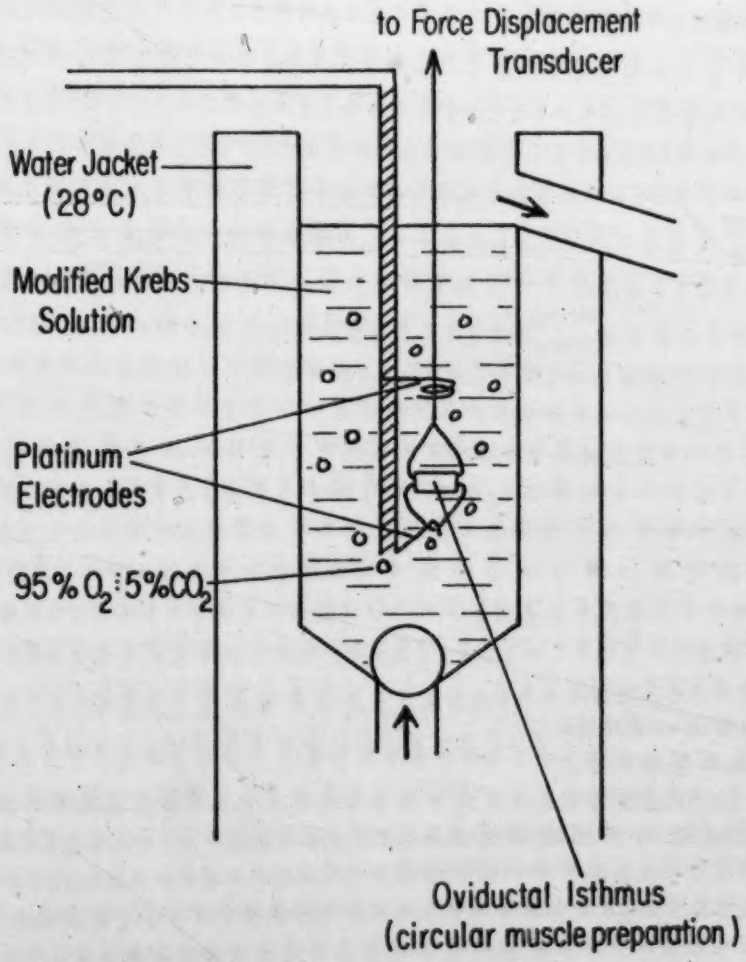

FIG. 5. Schematic illustration of contractility apparatus.

each experiment. Most of the remaining drugs were dissolved in the modified Krebs bathing solution.

17β-Estradiol was dissolved in absolute ethanol for subcutaneous injection. Progesterone and depo-estradiol were both dissolved in a small volume of acetone and made up in peanut oil for intramuscular injection. Human chorionic gonadotropin (HCG) was diluted with double-distilled water to yield an isotonic solution (mannitol present) at pH 7.2 for intravenous injection.

All chemicals were of analytical grade and were obtained from Fisher Scientific Company. These were dissolved in double-distilled water to make the modified Krebs solution (NaCl, 116 mM; KCl, 5 mM; $CaCl_2$, 1.25 mM; $MgCl_2$, 1.2 mM; NaH_2PO_4, 1.2 mM; $NaHCO_3$, 22 mM; D-glucose, 11.2 mM; with ascorbic acid, 0.1 mM and Na_2EDTA, 0.04 mM to prevent oxidation of amines).

IV Transmural Stimulation

A. Optimal Stimulation Characteristics

Initially, optimal characteristics for transmural stimulation were defined by monitoring responses of the circular isthmus to biphasic pulses of variable voltage, frequency, stimulus interval and stimulus train length. It was also desirable to establish that the contractile response to transmural stimulation resulted from the release of neurotransmitter from adrenergic nerve endings rather than from direct stimulation of oviduct smooth muscle. For this purpose, tetrodotoxin (5×10^{-7}M) which blocks action potential production in neurones, guanethidine (2×10^{-5}M) which inhibits transmitter release from adrenergic nerves, and phentolamine (9×10^{-6}M) which blocks α-adrenoceptors, were employed.

B. Frequency-Response Determinations

Responses of circular isthmus from each of the hormone treatment groups to increasing frequencies of transmural stimulation (supramaximal V, 1 msec pulse duration, 45 sec train, 1-24 Hz) were measured and expressed as % of the maximal response of the tissue. The frequencies required to produce 50% of the maximal response (EF_{50}) were determined by computer regression analysis of values between 20-80% on such frequency-response curves. The maximum tension developed was also noted for each group.

C. Blockade of Catecholamine Re-Uptake Processes and of β-Adrenoceptors

Tissue responses to transmural stimulation and relaxation times following stimulation were measured in the presence of cocaine (3×10^{-5}M) to block neuronal uptake, hydrocortisone (10^{-4}M) to block extraneuronal uptake, or propranolol (3.8×10^{-6}M) to block β-adrenoceptors.

Each response was expressed as a % of a control maximum response (24 Hz) to examine the effect of each agent on the magnitude of the response to transmural stimulation. EF_{50} values in the presence of these agents were determined by regression analysis of normalized frequency-response curves (i.e., each response was expressed as a % of the maximum response to 24 Hz). Relaxation was assessed by measuring the times taken to relax 30, 50, 70, 90 and 100% of the maximum response to 24 Hz.

V Adrenergic Agonists

A. Dose-Response Determinations and ED_{50} Calculations

Non-cumulative concentration-response curves to (-)-adrenaline, (-)-noradrenaline and (-)-phenylephrine were determined using at least 5 animals for each study. The responses to the various concentrations of agonist were expressed as a percentage of the maximal responses of the tissue (to 10^{-4}M adrenaline). Responses were normalized to eliminate

variability in the absolute tensions developed by tissues from different animals.

The potencies of adrenergic agonists were compared in terms of ED_{50} values; that is, the concentrations required to produce 50% of the maximum response. ED_{50} values were extrapolated by computer regression analysis of values between 20-80% on the log concentration vs response curves. Relative potencies of agonists were expressed as the ratio of the ED_{50} values and the potency of (-)-noradrenaline was arbitrarily chosen as 1.

The effects of various agents on the potencies of the adrenergic agonists were examined in dose-response determinations performed after a 20 min exposure to the drug. All drugs were present during the dose-response determination and comparison was made with a control tissue in each experiment.

B. Catecholamine Re-Uptake Processes

The potencies of (-)-adrenaline, (-)-noradrenaline and (-)-phenylephrine were compared in the presence of either cocaine (3×10^{-5}M), hydrocortisone (10^{-4}M) or cocaine + hydrocortisone.

C. β-Adrenoceptor Blockade

The potencies of the adrenergic agonists were determined in the presence of propranolol (3.8×10^{-6}M).

D. α-Adrenoceptor Activity

The potencies of (-)-phenylephrine in the presence of propranolol (3.8×10^{-6}M) and of (-)-adrenaline in the presence of cocaine (3×10^{-5}M), hydrocortisone (10^{-4}M) and propranolol (3.8×10^{-6}M) were measured to compare α-adrenoceptor activity in the various hormonal groups.

The order of potency of adrenergic agonists on α-adrenoceptors was also ascertained. Catecholamine re-uptake processes were blocked with

cocaine and hydrocortisone, and β-adrenoceptors were blocked with propranolol.

E. Characterization of β-Adrenoceptors

1. β-Adrenoceptor activity in estrous vs HCG + progesterone pretreated isthmus

β-Adrenoceptor activity in Estrous and HCG + Progesterone-pretreated tissues was determined by examining the (±)-isoprenaline antagonism of acetylcholine contractile responses (modification of Hodgson and Pauerstein, 1975). Since (±)-isoprenaline exhibits α-excitatory activity in this tissue at concentrations greater than 10^{-5}M, the equilibrated tissues were exposed to phenoxybenzamine (10^{-6}M) for 20 min to block α-adrenoceptors. Cocaine (3×10^{-5}M) and hydrocortisone (10^{-4}M) were included in the Krebs solution to, respectively, block neuronal and extraneuronal uptake of catecholamines.

Contractile responses to a maximal dose of acetylcholine (5×10^{-4}M) were elicited every 15 min. This time schedule was selected to avoid tissue desensitization. Then, 2 min before an acetylcholine injection, various doses of (±)-isoprenaline (2×10^{-7}M - 10^{-4}M) were administered. The % inhibition of the acetylcholine response was calculated by comparing the magnitude of the response in the presence of (±)-isoprenaline to control responses before and after. The maximum inhibitory responses as well as ID_{50} values were compared between hormone treatment groups.

2. Order of agonist potency on β-adrenoceptors

A series of experiments as described above was completed for (-)-adrenaline, (-)-noradrenaline and (±)-salbutamol antagonism of the acetylcholine response in Estrous and HCG + Progesterone-pretreated tissues. The orders of agonist potency on β-adrenoceptors were ascertained. The maximum inhibitory effects of the agonists were also noted.

3. Non-specific inhibition of the acetylcholine response

Dose-response curves for papaverine inhibition of the cholinergic contractile response were similarly studied in the two hormone groups. The ID_{50} values and maximum inhibitory responses were compared between groups.

4. pA_2 values for competitive β-antagonists

A similar experimental procedure was employed in the determination of pA_2 values for competitive antagonists of β-adrenoceptor activity in HCG + Progesterone pretreated tissues. Two doses of (±)-isoprenaline (x, 2x) were administered and the percentage inhibitions of the acetylcholine response were calculated. The tissues were then exposed to various concentrations of the β-antagonists (propranolol or practolol, a specific β_1-antagonist). The 2x dose of (±)-isoprenaline was repeated in the presence of the antagonist. In every experiment each tissue served as its own control and one tissue was not exposed to antagonist. The pA_2 value was determined as the $-\log_{10}$ of the concentration of antagonist which reduced the effect of the 2x dose of the agonist to that of x. A maximal inhibitory concentration of (±)-isoprenaline (10^{-4}M) was employed to demonstrate that the competitive antagonists did not reduce the maximum effect.

VI Statistical Analysis

The results were expressed as the mean ± standard error of the mean. The unpaired Student's two-tailed t-test was employed to make comparative assessments. 95% was chosen as an acceptable level of significance.

RESULTS

I Transmural Stimulation

A. Optimal Stimulation Characteristics

Optimal characteristics were determined for the transmural stimulation of the circular smooth muscle of the oviductal isthmus. Several stimulus parameters were varied such that maximal tissue contractile responses were obtained.

No significant improvement of the response to 8 Hz (35V) was obtained at greater than 1 msec pulse duration; hence, a pulse duration setting of 1 msec was employed in all subsequent experiments.

The voltage of the electrical stimulation was varied from 11-42 V. The maximal voltage was determined to be 35 V and a supramaximal setting (42V) was selected for use. Frequency-response determinations (2-24 Hz) revealed a maximum contractile response at about 20 Hz.

Three minutes was found to be the minimum time lapse between stimulations which did not cause reduction of the second response height. In subsequent studies, therefore, five minutes was used as a convenient stimulus interval. The stimulus train length was varied from 5-45 sec. The full magnitude of the tissue response was expressed after 30 sec of stimulation; hence, 45 sec was selected for use.

These findings are presented in Figure 6. As a result of this exercise, the following stimulation characteristics were used routinely in subsequent frequency-response curve determinations: 1 msec pulse duration, supramaximal voltage, 5 min stimulus interval and 45 sec train length.

Tetrodotoxin which blocks action potential production in neurones, guanethidine which inhibits transmitter release from adrenergic nerve endings, and phentolamine which blocks α-adrenoceptors, were employed to establish that the response to transmural stimulation resulted from the release of noradrenaline from adrenergic nerve endings. Each agent was

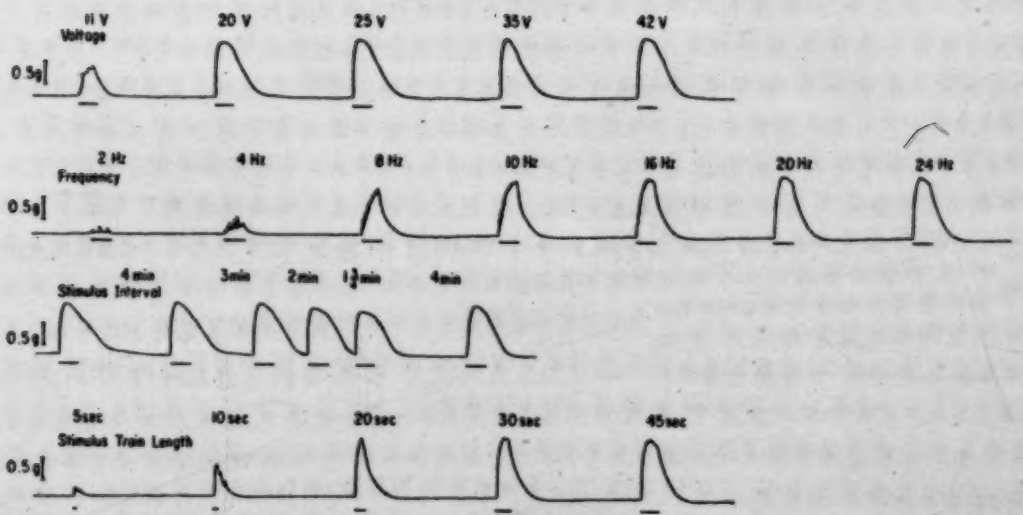

FIG. 6 Effect of voltage, frequency, stimulus interval and stimulus train length on the response of the isthmic circular muscle of rabbit oviduct to transmural stimulation. (____, period of transmural stimulation).

successful in abolishing contractions of the circular isthmus to electrical stimulation. It is important to note that the tetrodotoxin- and guanethidine-treated tissues (i.e., with presynaptic adrenergic blockade) remained responsive to exogenous noradrenaline while phentolamine blockade of α-adrenoceptors abolished the response to exogenous noradrenaline.

These results are illustrated in Figure 7 and indicate that the contractile response to transmural stimulation was indeed caused by activation of α-receptors by noradrenaline released from adrenergic nerves.

B. Frequency-Response Determinations

The % of maximum response of rabbit isthmus is plotted against increasing stimulus frequency in Figure 8. The frequencies required to produce a 50% of maximum response were significantly different (Estrus : 3.7 ± 0.9 Hz, n = 5; HCG + Progesterone : 8.1 ± 0.6 Hz, n = 10; HCG + Estrogen : 5.3 ± 0.4, n = 9).

Transmural stimulation at 24 Hz was maximal for all tissues, but the maximum tension developed was significantly greater in the Estrous Group than in the HCG-treated Groups II and III (Estrus : 0.9 ± 0.1 g, n = 17; HCG + Progesterone : 0.5 ± 0.1 g, n = 16; HCG + Estrogen : 0.6 ± 0.1 g, n = 14).

C. Effect of Blockade of Catecholamine Reuptake Processes and of β-Adrenoceptor Blockade

1. Magnitude of responses to transmural stimulation

The influence of cocaine (3×10^{-5}M) on isthmic contractile responses to transmural stimulation is illustrated in Figure 9. Responses to increasing stimulus frequencies are plotted as % of the maximum determined in a previous control stimulation at 24 Hz. Cocaine significantly reduced the response to frequencies greater than 6 Hz in all hormone groups, but

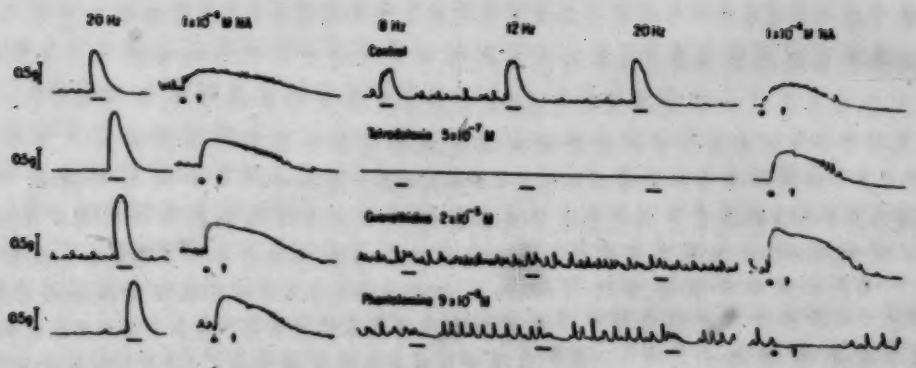

FIG. 7 Effect of tetrodotoxin (5×10^{-7}M), guanethidine (2×10^{-5}M) and phentolamine (9×10^{-6}M) on the responses of the isthmic circular muscle of rabbit oviduct to transmural stimulation and to exogenous noradrenaline (10^{-4}M). (____, period of transmural stimulation; •, administration of adrenergic agonist; †, wash).

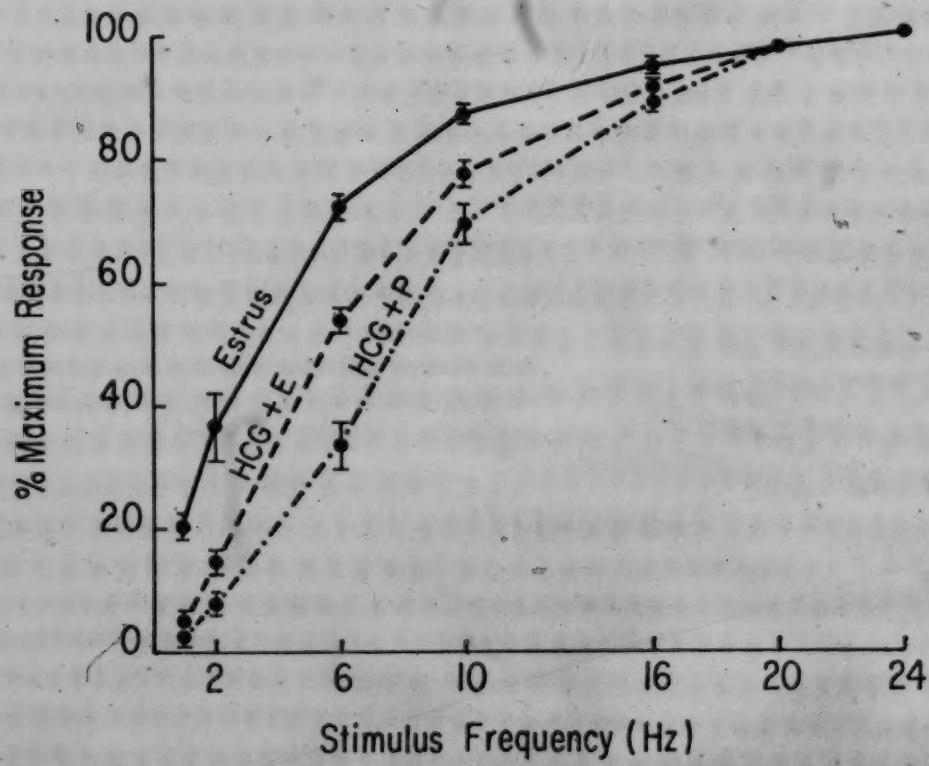

FIG. 8 Effect of hormone pretreatment on frequency-response curves of rabbit oviductal isthmus. Each point represents the mean % maximum response ± standard error (n = 10-13).

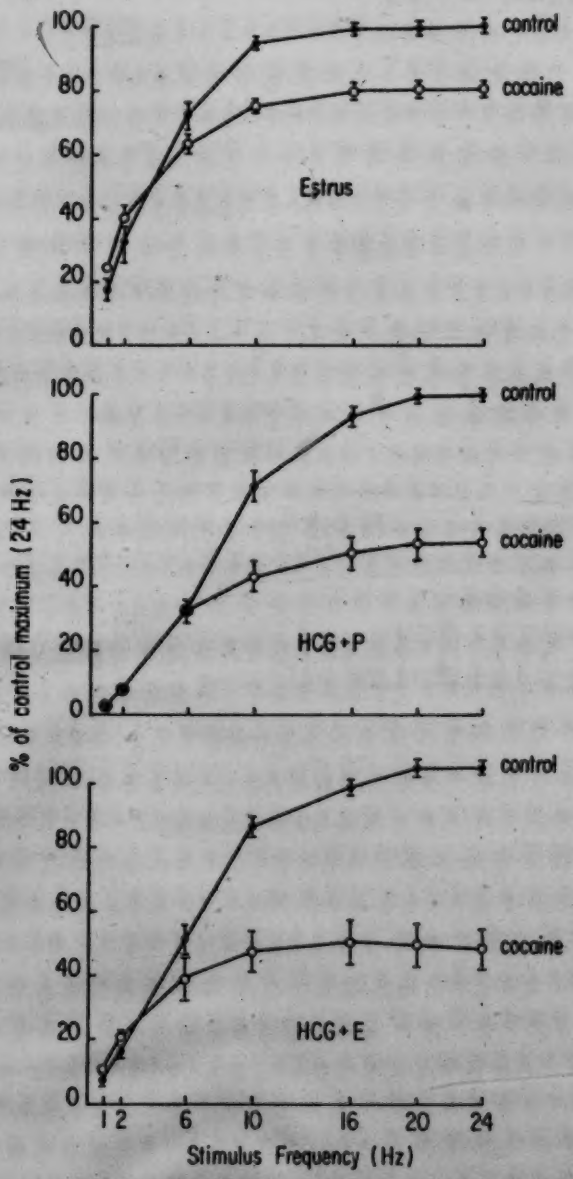

FIG. 9 Effect of cocaine (3×10^{-5}M) on contractile responses of rabbit isthmus to transmural stimulation in Estrous, HCG + Progesterone and HCG + Estrogen tissues. Each point represents the mean % of control maximum response ± standard error (n = 10-13).

produced no significant effect at lower frequencies. In the presence of cocaine, the maximum responses of Estrous group tissues and of HCG-treated tissues were reduced to 80% and 50%, respectively, of the control maxima.

Hydrocortisone (10^{-4}M) had no effect on the maximum responses to transmural stimulation.

Propranolol (3.8×10^{-6}M) slightly reduced the maximum response to transmural stimulation in the HCG-treated Groups II and III but had no effect in Group I : Estrus (see Fig. 10).

2. Frequencies required to produce a 50% of maximum response

The effects of blockade of catecholamine reuptake processes and of β-adrenoceptor blockade on the stimulus frequency required to produce a 50% of maximum response (EF_{50}) are presented in Table I. Cocaine (3×10^{-5}M) reduced the EF_{50} from control values in all hormone groups. However, neither hydrocortisone (10^{-4}M) nor propranolol (3.8×10^{-6}M) caused a shift in the EF_{50} values.

3. Relaxation times following transmural stimulation

The effects of cocaine (3×10^{-5}M) on the relaxation of the isthmic circular muscle preparations following transmural stimulation at 24 Hz are presented in Figure 11. Cocaine significantly prolonged relaxation times in all hormone groups.

Hydrocortisone (10^{-4}M) alone had no effect on relaxation times following stimulation, however, in Estrous tissues, relaxation in the presence of cocaine + hydrocortisone was significantly more prolonged than in the presence of cocaine alone. This is illustrated in the top diagram of Figure 11.

Propranolol (3.8×10^{-6}M) had no effect on relaxation times following transmural stimulation.

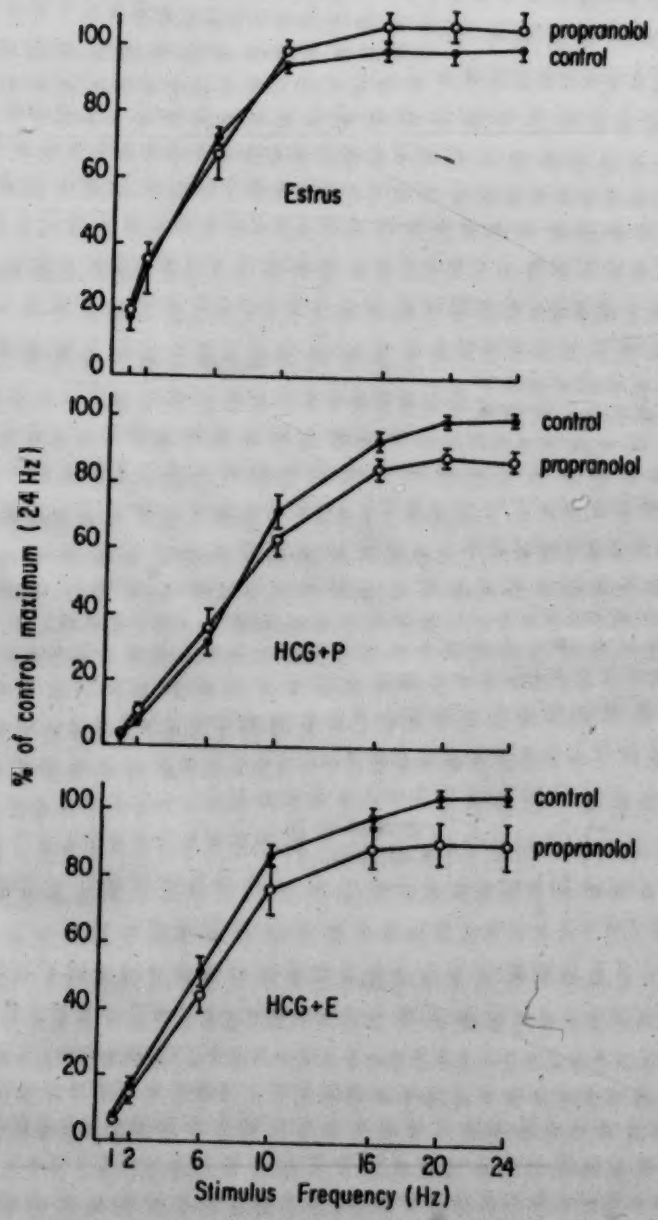

FIG. 10 Effect of propranolol on contractile responses of rabbit isthmus to transmural stimulation in Estrous, HCG + Progesterone and HCG + Estrogen tissues. Each point represents the mean % of control maximum response ± standard error (Control, n = 10-13; 3.8×10^{-6}M propranolol, n = 5).

TABLE I Effect of blockade of catecholamine reuptake processes and of β-adrenoceptor blockade on the stimulus frequency required to produce a 50% of maximum response (EF_{50}) in rabbit oviductal isthmus.

EF_{50} ± standard error of the mean (n)

Treatment	Estrus	HCG + Progesterone	HCG + Estrogen
Control	3.7 ± 0.9 (5)	8.1 ± 0.6 (10)	5.3 ± 0.4 (9)
Propranolol	4.7 ± 0.8 (4)	7.0 ± 0.7 (8)	5.9 ± 0.5 (5)
Cocaine	2.4 ± 0.1 (6)	5.1 ± 0.4 (10)	2.9 ± 0.3 (9)
Hydrocortisone	3.3 ± 0.8 (6)	8.6 ± 0.4 (5)	5.9 ± 0.9 (5)
Cocaine + Hydrocortisone	2.4 ± 0.5 (6)	4.5 ± 0.7 (4)	2.5 ± 0.6 (5)

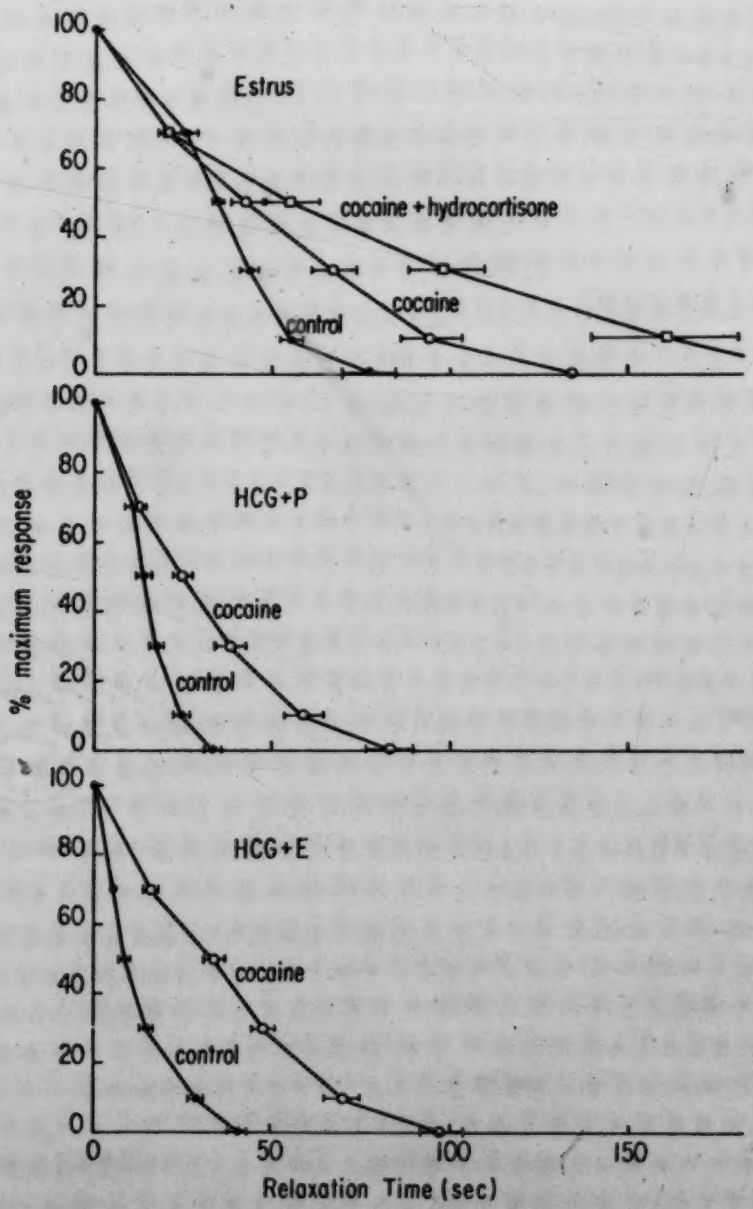

FIG. 11 Effect of cocaine (3×10^{-5}M) on relaxation of oviductal isthmus following transmural stimulation in Estrous, HCG + Progesterone and HCG + Estrogen tissues. Each point represents the mean % maximum response ± standard error (n = 7-9). The effect of cocaine + hydrocortisone (10^{-4}M) on relaxation times in Estrous tissues is illustrated in the top graph (n = 5).

II Adrenergic Agonists

A. Potency of Adrenegic Agonists

(-)-Adrenaline was more potent than (-)-noradrenaline or (-)-phenylephrine in all tissues. In Group I : Estrus and Group III : ECG + Estrogen, (-)-noradrenaline was equipotent with (-)-phenylephrine, but, in Group II : HCG + Progesterone, (-)-noradrenaline was significantly less potent. The potency of each agonist between groups did not vary for (-)-adrenaline or (-)-phenylephrine, but in HCG + Progesterone-pretreated tissues (-)-noradrenaline was significantly less potent than in the Estrous or HCG + Estrogen groups. The results are presented in terms of ED_{50} values in histogram form in Figure 12. Tables presenting details of the results for adrenergic agonists (mean ED_{50}, standard error, number of experiments) are contained in the Appendix).

The maximum tension developed to (-)-adrenaline (10^{-4}M) was significantly greater in the Estrus group than in the HCG-treated Groups II and III (Estrus : 0.9 ± 0.1 g, n = 14; HCG + Progesterone : 0.6 ± 0.1 g, n = 14; HCG + Estrogen : 0.6 ± 0.1 g, n = 14).

B. Effect of Blockade of Catecholamine Re-Uptake Processes on Agonist Potency

Cocaine (3×10^{-5}M) potentiated (-)-noradrenaline more than (-)-adrenaline in all tissues and had no effect on the potency of (-)-phenylephrine. The potencies of (-)-noradrenaline in the presence and absence of cocaine for each of the hormone groups are presented in Figure 13.

Cocaine eliminated the difference in potency between (-)-noradrenaline and (-)-phenylephrine in HCG + Progesterone-pretreated tissues.

While cocaine potentiated (-)-noradrenaline in all treatment groups it did not abolish the between-group potency differences; that is, the

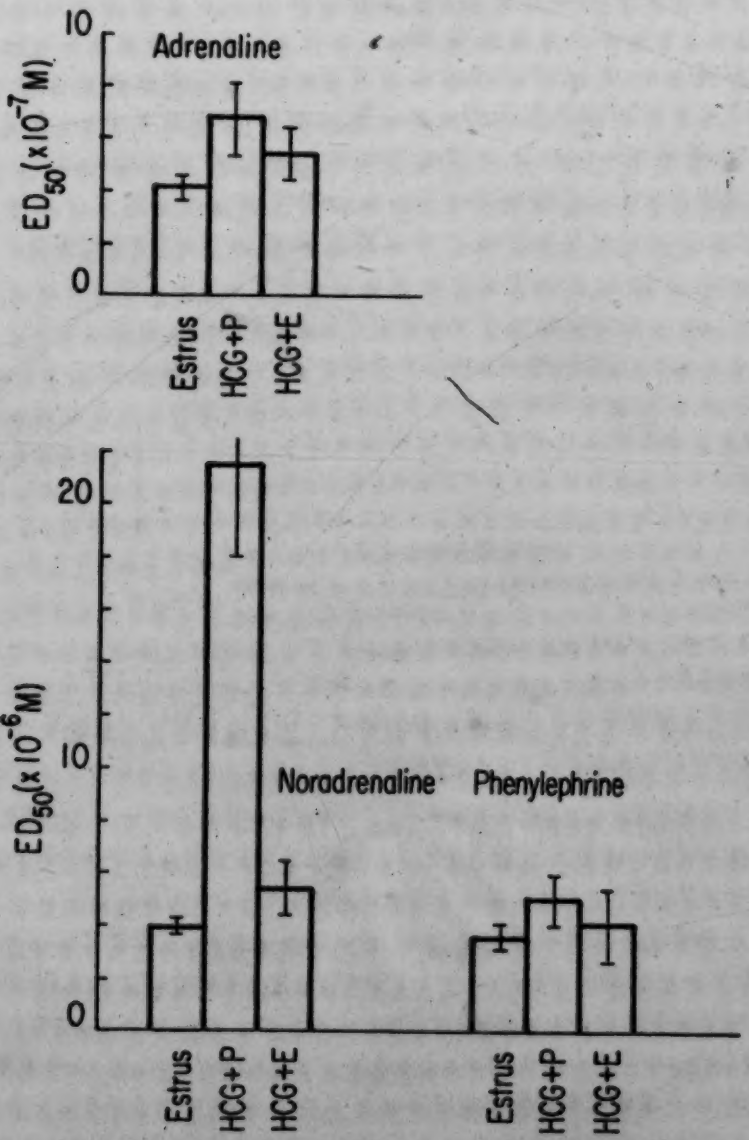

FIG. 12 Potency of (-)-adrenaline, (-)-noradrenaline and (-)-phenylephrine in Estrous, HCG + Progesterone and HCG + Estrogen tissues. Each histogram represents the mean ED_{50} ± standard error (n = 7-12).

ED_{50} for (-)-noradrenaline in the presence of cocaine remained greater in Group II : HCG + Progesterone (5×10^{-6}M) than in Group I : Estrus (5×10^{-7}M) or Group III : HCG + Estrogen (1×10^{-6}M).

Agonist potencies were not affected by hydrocortisone (10^{-4}M) alone, and ED_{50} values in the presence of cocaine + hydrocortisone were not significantly different from those with cocaine alone. This is illustrated for (-)-noradrenaline in Figure 13. In the presence of cocaine + hydrocortisone (i.e., blockade of neuronal and extraneuronal uptake), the order of potency was (-)-adrenaline > (-)-noradrenaline > (-)-phenylephrine.

C. Effect of β-Adrenoceptor Blockade on Agonist Potency

Propranolol (3.8×10^{-6}M) had no effect on the potencies of adrenaline or phenylephrine. However, Figure 14 illustrates that propranolol potentiated noradrenaline only in HCG + Progesterone tissues and eliminated between-group potency differences; that is, the ED_{50} values for noradrenaline in the presence of propranolol were not significantly different. (Estrus : 6×10^{-6}M; HCG + Progesterone : 8×10^{-6}M; HCG + Estrogen : 5×10^{-6}M).

D. α-Adrenoceptor Sensitivity

α-Adrenoceptor sensitivity did not vary between groups since the potency of (-)-phenylephrine in the presence of propranolol (3.8×10^{-6}M) and the potency of (-)-adrenaline in the presence of cocaine + hydrocortisone + propranolol were not altered by any of the hormonal treatments. This is illustrated in Figure 15.

The order of potency for the adrenergic agonists on α-adrenoceptors (i.e., in the presence of cocaine + hydrocortisone + propranolol) was determined to be (-)-adrenaline > (-)-noradrenaline > (-)-phenylephrine. In

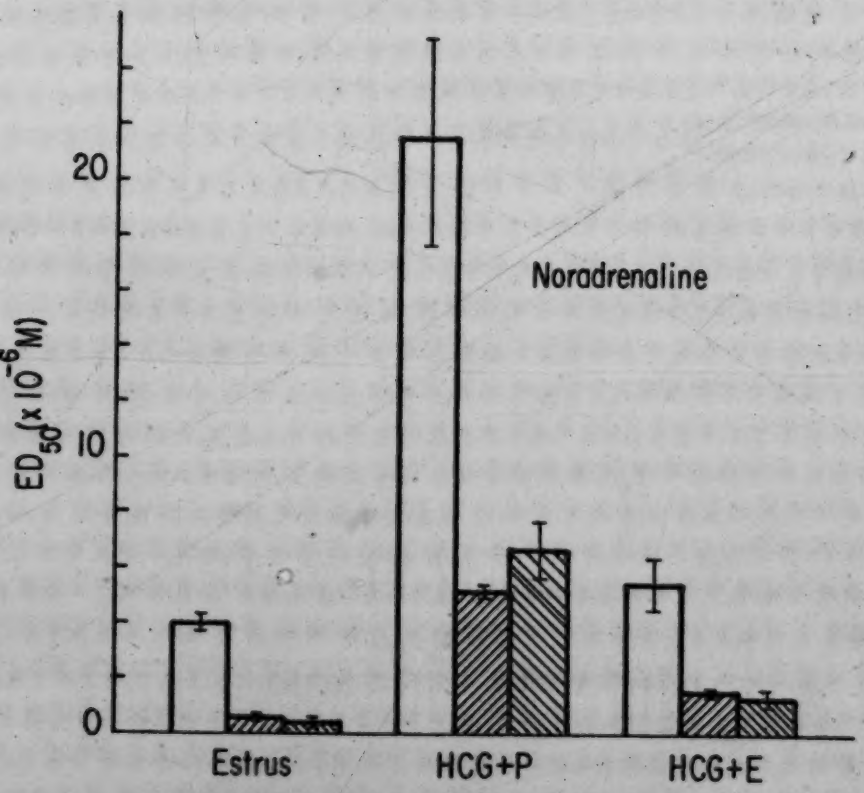

FIG. 13 Effect of blockade of neuronal and extraneuronal uptake on the potency of (-)-noradrenaline in Estrous, HCG + Progesterone and HCG + Estrogen-treated rabbit isthmus. Each histogram represents the mean ED_{50} ± standard error (Control □ , n = 9-12; 3 x 10^{-5}M cocaine ▨, n = 8-12; cocaine + 10^{-4}M hydrocortisone ▧ , n = 5).

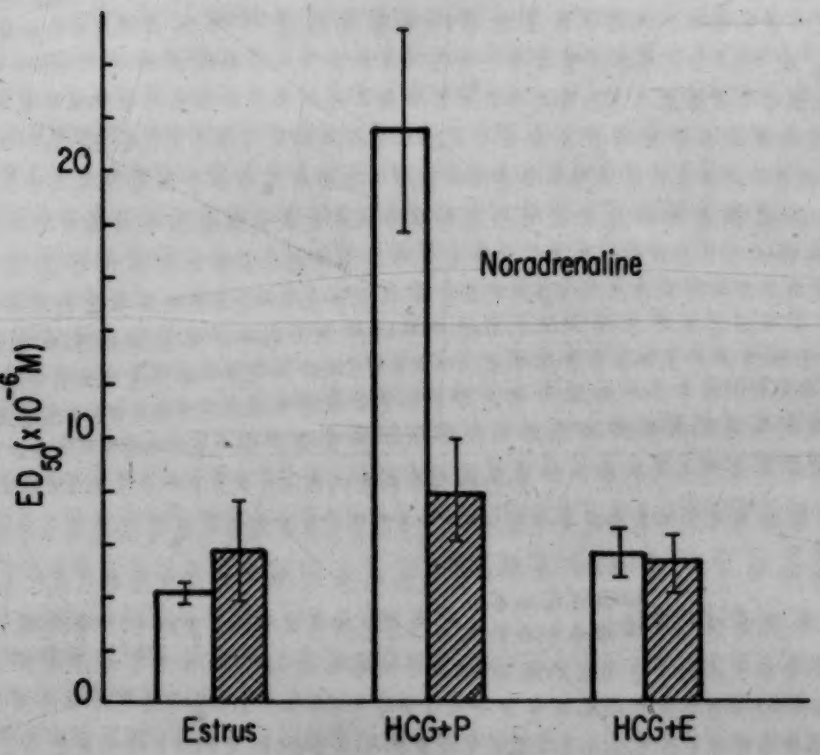

FIG. 14 Effect of β-adrenoceptor blockade on the potency of (-)-noradrenaline in Estrous, HCG + Progesterone and HCG + Estrogen-treated rabbit isthmus. Each histogram represents the mean ED_{50} ± standard error (Control □ , n = 9-12; 3.8×10^{-6}M propranolol ▨ , n = 5-6).

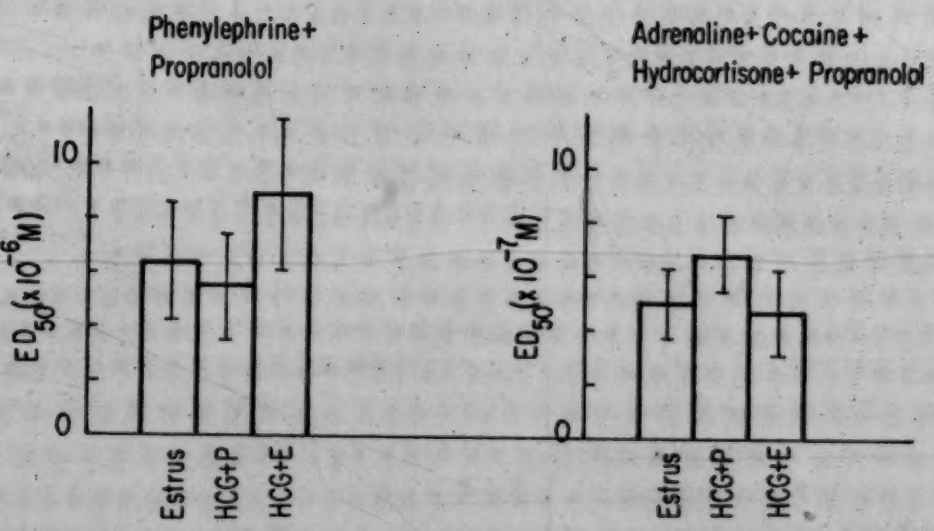

FIG. 15 α-Adrenoceptor sensitivity of rabbit oviductal isthmus in Estrous, HCG + Progesterone and HCG + Estrogen tissues. Each histogram represents the mean ED_{50} ± standard error for (-)-phenylephrine + propranolol (n = 5-6) and for (-)-adrenaline + cocaine + hydrocortisone + propranolol (n = 5).

Table II the relative potencies for the adrenergic agonists on α-adrenoceptors in oviductal isthmus are compared with values for other rabbit tissues.

E. Characterization of β-Adrenoceptors

1. β-Adrenoceptor activity in Estrous vs HCG + Progesterone-pretreated isthmus

(±)-Isoprenaline antagonism of the acetylcholine-elicited contractile response was studied to provide a measure of β-adrenoceptor activity in Estrous vs HCG + Progesterone-pretreated isthmus. Figure 16 plots the % inhibition of the acetylcholine response against increasing (±)-isoprenaline concentration. In Progesterone-pretreated tissues, the β-agonist maximally inhibited the contractile response to acetylcholine by about 90%, while in Estrous tissues, (±)-isoprenaline only inhibited the acetylcholine response by about 30%. Propranolol blocked the effect of (±)-isoprenaline in both groups.

The magnitude of the contractile response to acetylcholine (5×10^{-4}M) was significantly greater in Estrous tissues (0.42 ± 0.05 g, n = 7) than in HCG + Progesterone-pretreated tissues (0.27 ± 0.03 g,, n=13). In spite of this, the absolute magnitude of the (±)-isoprenaline inhibition of the acetylcholine response was still greater in the HCG + Progesterone group (0.27 g x 0.90 = 0.24 g) than in the Estrous group (0.42 g x 0.30 = 0.13 g).

Normalized curves for the inhibitory effect of (±)-isoprenaline are presented in Figure 17; that is, each inhibitory response is expressed as a % of the maximum inhibitory response for each treatment group and is plotted against (±)-isoprenaline concentration. When expressed in this manner, the ID_{50} values do not differ between the Estrous (3.4×10^{-7}M) and HCG + Progesterone (5.2×10^{-7}M) groups. Thus, while the maximum inhibitory effect was greater in the HCG + Progesterone group, the potency

TABLE II Relative potencies of adrenergic agonists on α-adrenoceptors in various rabbit tissues.

Rabbit Tissue	Relative Potencies		
	(-)-Adrenaline	(-)-Phenylephrine	(-)-Noradrenaline
*Thoracic aorta	1.2	0.2	1
*Duodenum	2.0	0.25	1
*Stomach fundus	1.0	0.25	1
Oviductal isthmus (estrus)	1.1	0.2	1

*from Furchgott, 1972, p. 326.

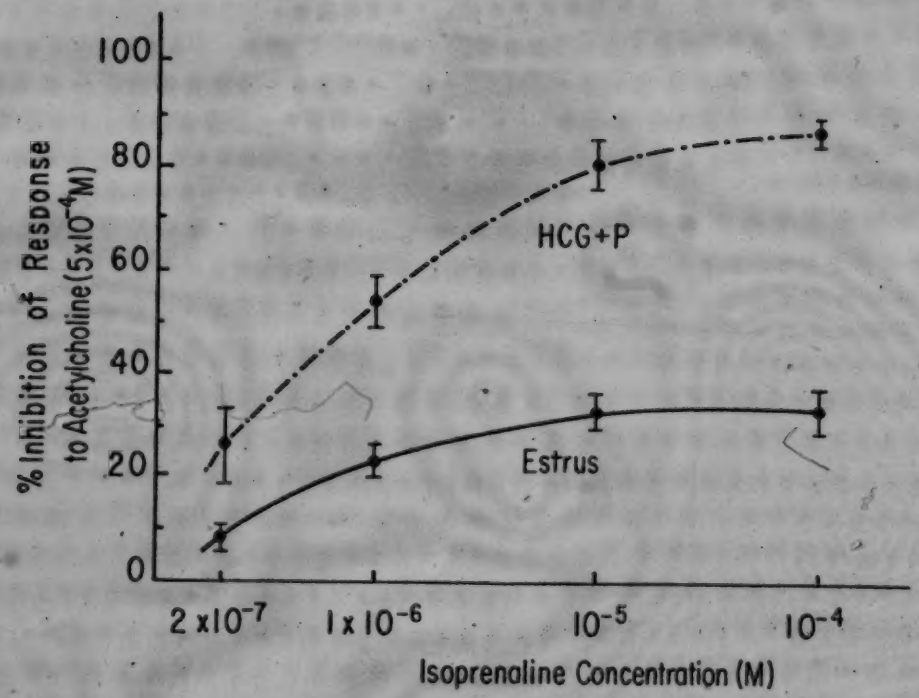

FIG. 16 β-Adrenoceptor activity of rabbit oviductal isthmus in Estrous and HCG + Progesterone-pretreated isthmus. Each point represents the mean ± standard error for the % inhibition of the acetylcholine contractile response by (±)-isoprenaline. (Estrus ●——●, n = 8; HCG + Progesterone ●-.-.-●, n = 6).

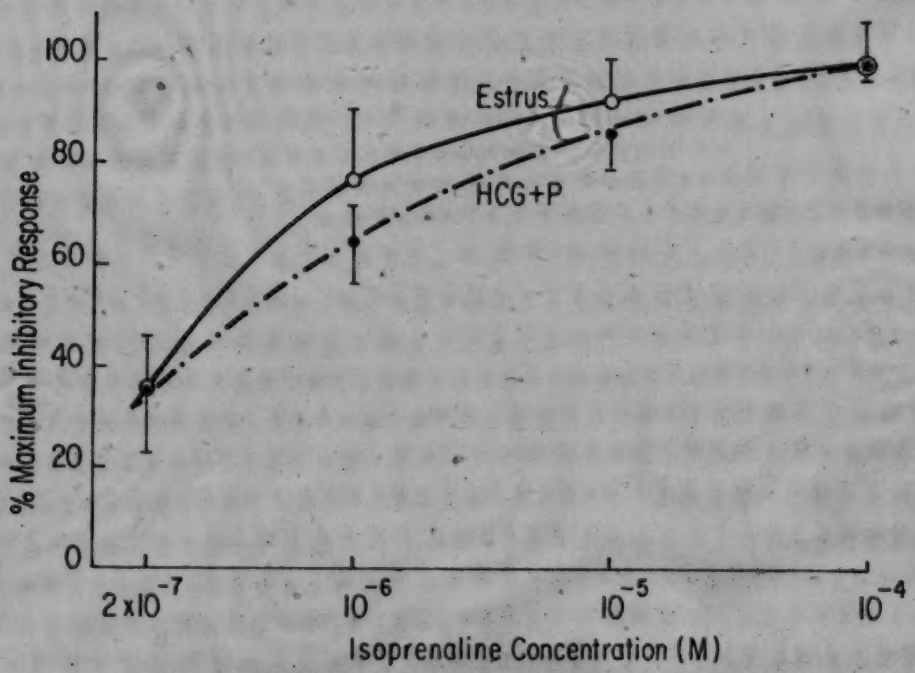

FIG. 17 Normalized curves for the inhibitory effect of (±)-isoprenaline on the acetylcholine response in Estrous and HCG + Progesterone-pretreated tissues. Each inhibitory response is expressed as a % of the maximum inhibitory response for that hormone group ± standard error. (Estrus o——o, n = 8; HCG + Progesterone •-.-.-•, n = 6).

of (±)-isoprenaline on β-adrenoceptors did not vary.

2. Order of agonist potency on β-adrenoceptors

A series of experiments for the antagonism of the acetylcholine response by (-)-adrenaline, (-)-noradrenaline, (±)-isoprenaline and (±)-salbutamol was completed. The order of agonist potency on β-adrenoceptors was (±)-isoprenaline ≥ (-)-noradrenaline > (-)-adrenaline >> (±)-salbutamol.

Table III lists the relative potencies for the adrenergic agonists on β-adrenoceptors in oviductal isthmus along with comparable values for other rabbit tissues.

3. Inhibition of the acetylcholine response by a specific β_2-agonist

(-)-Adrenaline and (-)-noradrenaline produced maximum inhibitory effects of the same order of magnitude as (±)-isoprenaline. However, (±)-salbutamol, a specific β_2-agonist failed to achieve such a maximum effect, and at 10^{-4}M elicited only 40-50% of the maximum inhibitory response to (±)-isoprenaline. This is illustrated in Figure 18.

4. Non-specific inhibition of the acetylcholine response

The results from the study of papaverine inhibition of the acetylcholine response are presented in Figure 19. Papaverine exhibited identical dose-response curves in both hormone groups : the maximum inhibitory response was 100% in both groups, and ID_{50} values (Estrus : 1.3×10^{-5}M; HCG + Progesterone : 1.2×10^{-5}M) were not significantly different.

5. pA_2 values for competitive β-antagonists

The pA_2 value for propranolol was 8.3 and for practolol, a specific β_1-antagonist, 5.3. Neither antagonist was found to reduce the maximum inhibitory effect of (±)-isoprenaline.

The pA_2 values for propranolol and practolol against (±)-isoprenaline are listed in Table IV along with comparable values from other rabbit tissues.

TABLE III Relative potencies of adrenergic agonists on β-adrenoceptors in various rabbit tissues.

Rabbit Tissue	Relative Potencies			
	(-)-Isoprenaline	(-)-Adrenaline	(-)-Noradrenaline	(±)-Salbutamol
*Thoracic aorta	130	65	1	
*Left atrium	3.5	0.5	1	
*Small intestine	1.5	0.2	1	
*Stomach fundus	2.5	1.2	1	
Oviductal isthmus (estrus)	1.5	0.8	1	0.2
(HCG + P)	1.0	0.4	1	0.1

*from Furchgott, 1972, p. 322.

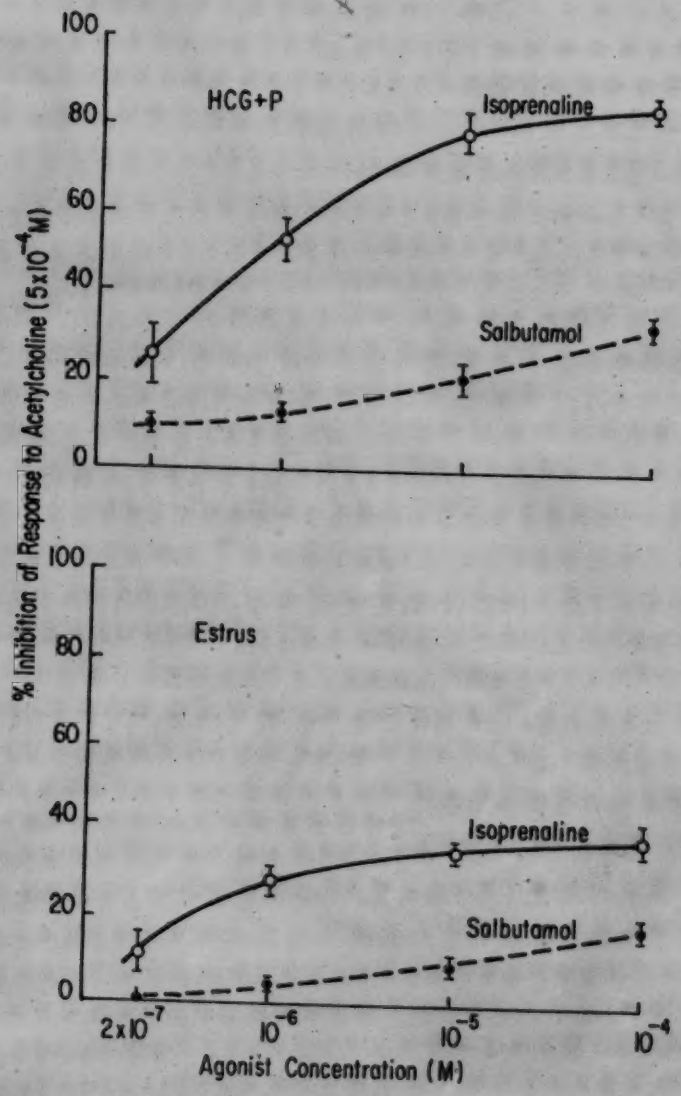

FIG. 18 Comparison of the inhibitory effects of (±)-salbutamol (β_2-agonist) and (±)-isoprenaline on the response to acetylcholine (5×10^{-4}M) in Estrous and HCG + Progesterone-pretreated rabbit isthmus. Each point represents the mean % inhibition ± standard error for increasing agonist concentrations. ((±)-Isoprenaline o——o, n = 6; (±)-salbutamol •---•, n = 4-6).

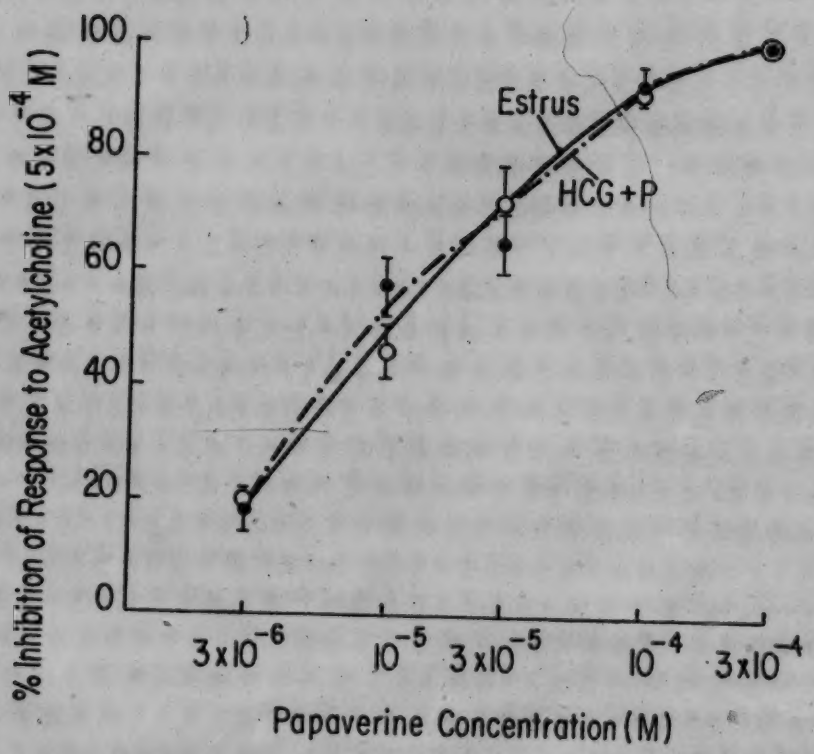

FIG. 19 Dose-response curves for the inhibition of the acetylcholine response by a non-specific smooth muscle relaxant in Estrous and HCG + Progesterone-pretreated rabbit isthmus. Each point represents the mean % inhibition ± standard error for increasing papaverine concentrations. (Estrus o——o, n = 5; HCG + Progesterone •-.-.-•, n = 5).

TABLE IV pA_2 values for competitive antagonists of β-adrenoceptors in various rabbit tissues.

Rabbit Tissue	β-Blocking Agent		β-Receptor Sub-Type
	Propranolol	Practolol	
*Thoracic aorta	8.98	3.89	β_2
*Stomach fundus	6.89	<3.50	β_2
*Right atrium	8.8	6.91	β_1
Oviductal isthmus (HCG + P)	8.3	5.3	

*from Furchgott, 1972, p. 322.

DISCUSSION

I Discussion of Results

The present experiments were performed in a modified Krebs solution at 28°C. This temperature was selected on the basis of previous findings (Paton and Johns, 1975b) which demonstrated spontaneous desensitization of rabbit oviductal isthmus at 37°C, to noradrenaline and, to an even greater degree, to transmural stimulation. Indomethacin prevented this desensitization as did lower tissue bath temperature. This work suggested that the tissue desensitization was due to the spontaneous liberation, at 37°C, of prostaglandins which inhibit oviductal contractility. The Ca^{++} concentration in the Krebs solution was 1.25 mM. This concentration was selected in order to reduce spontaneous contractility.

We have investigated the effects of transmural stimulation and of adrenergic agonists on the contractility of isthmic circular muscle of rabbits in estrus and after treatment with sex hormone schedules known to disrupt normal transport. In all tissues, transmural stimulation elicited contractile responses which were caused by activation of α-receptors by noradrenaline released from adrenergic nerves. The maximum tension developed to transmural stimulation and to adrenaline was greater in Estrous tissues than in the HCG-treated Groups II and III.

Higgs and Moawad (1974) have demonstrated that maximum responses to adrenergic agonists and to Ca^{++} were similarly depressed in progesterone-dominant tissues and suggested that progesterone might interfere with the excitation-contraction sequence at some point beyond receptor activation. The possibility of an adrenergic component for the hormonal modification of oviduct function in ovum transport is not eliminated by their work, however, since the dose-response curve for noradrenaline

was shifted to the right with progesterone treatment while the dose-response curve for Ca^{++} was not affected.

It has been demonstrated that use of HCG as an ovulatory stimulus results in increased levels of plasma progesterone (Shaikh and Harper, 1972; Spilman and Wilks, 1976; Wu, 1977). Thus, the HCG treatment itself, through altered progesterone levels, may be responsible for the reduced magnitude of contractile responses in the present study. The finding that the frequency required to produce a 50% of maximum response was significantly greater in the HCG-treated Groups II and III than in Estrous tissues parallels the shift to the right that Higgs and Moawad (1974) observed for noradrenaline, but not for Ca^{++}, in progesterone-dominant tissues.

While cocaine (3×10^{-5}M) produced supersensitivity to exogenous (-)-noradrenaline, this concentration reduced the magnitude of tissue responses to transmural stimulation. This failure of cocaine to potentiate responses to transmural stimulation may be related to its local anaesthetic action causing a reduction in transmitter release from adrenergic nerves (Hukovic and Muscholl, 1962). Also, since cocaine inhibits the neuronal uptake of noradrenaline, released noradrenaline may persist to effect a presynaptic α-receptor mediated inhibition of further transmitter release (Enero et al., 1972; Langer, 1974).

This reasoning is supported by the finding that propranolol, which also produces a certain degree of local anaesthetic action (Lemmer and Saller, 1974; Stjarne and Brundin, 1976), reduced the maximum response at least in the HCG-treated groups. Further, hydrocortisone showed no inhibition of the maximum responses. Since

extraneuronal uptake is not of major significance to noradrenaline inactivation in this tissue (Johns and Paton, 1976), hydrocortisone would not produce a build-up of noradrenaline that might have pre-synaptic inhibitory consequences.

The present report of reduced tissue responses to transmural stimulation by cocaine agrees with the findings of Johns and Paton (1975). Recently, however, cocaine (3×10^{-5}M) has been reported to significantly increase the response of rabbit isthmus, particularly at frequencies between 5-10 Hz (Kennedy and Marshall, 1977). Some explanation for these contradictory results may be found in the different experimental procedures employed: Kennedy and Marshall used extrinsic nerve stimulation rather than transmural stimulation and used ovariectomized + hormone-supplemented groups rather than the hormone groups described in this study.

The prolongation of the relaxation times by cocaine indicates the importance of neuronal uptake in determining the duration of exposure of the receptors to released noradrenaline (Costa, Furness and Dawson, 1975). Previous studies (Johns and Paton, 1975, 1976) have shown that neuronal uptake is, indeed, the principal route of inactivation for noradrenaline in the ampulla and isthmus of rabbit oviduct. Cocaine + hydrocortisone significantly prolonged relaxation times in Estrous tissues more than with cocaine alone. Therefore, extraneuronal uptake may be of some significance when neuronal uptake mechanisms are blocked; i.e., when a large concentration of neuro-transmitter may be realized at the extraneuronal uptake sites.

It has been demonstrated by various workers (see Introduction Part VII) that the α-adrenoceptors in the rabbit oviduct are

excitatory while the β-adrenoceptors mediate relaxation. Both α- and β-activity were demonstrated in the isthmic smooth muscle preparations in this study. α-Adrenoceptor activity predominated in all hormonal groups since adrenaline and noradrenaline, which act on both receptor types, elicited contractile responses which were blocked by phentolamine or phenoxybenzamine.

A major observation of the present study of the action of adrenergic agonists on the contractility of rabbit isthmic circular muscle was the decreased potency of noradrenaline in HCG + Progesterone-pretreated tissues. Modifications in catecholamine re-uptake processes and α- and β-adrenoceptor sensitivity were investigated as possible explanations for the decreased potency in the HCG + Progesterone group. These were studied with an appreciation that reduced responsiveness of the circular isthmus might represent contractility changes related to the acceleration of ovum transport by the HCG + Progesterone pretreatment.

Blockade of neuronal uptake of catecholamines with cocaine potentiated noradrenaline more than adrenaline in all groups and had no effect on the potency of phenylephrine. This finding correlates with the affinity of the various adrenergic agonists for the neuronal uptake system (Iversen, 1967).

Blockade of extraneuronal uptake with hydrocortisone had no effect on agonist potency, and ED_{50} values in the presence of cocaine + hydrocortisone were not significantly different from those with cocaine alone. This is in accord with our findings for the effect of hydrocortisone on oviduct responses to transmural stimulation and further substantiates the theory that extraneuronal uptake is of little

importance in the inactivation of noradrenaline in rabbit oviduct (Johns and Paton, 1975, 1976).

Blockade of catecholamine uptake processes did not eliminate between-group noradrenaline potency differences: noradrenaline was still less potent in HCG + Progesterone tissues in the presence of cocaine, hydrocortisone or both agents. Modifications in catecholamine uptake mechanisms are, therefore, probably not important in contractility changes that may be related to the acceleration of ovum transport.

A second possibility which might explain the depression of noradrenaline potency by progesterone was that receptor sensitivity might be altered. A deactivation of α-adrenoceptors or, alternatively, an activation of β-adrenoceptors with progesterone treatment might explain the observation. The relative sensitivity of the two adrenergic receptor types mediating excitatory or inhibitory tissue responses may be of importance in oviduct motility changes related to the modification of ovum transport.

α-Receptor sensitivity did not vary between the hormone groups in this study. This finding is not in accord with the conclusions of Hunter and Kendle (1974) nor of Heilman et al. (1976) who have attributed the effect of progesterone to a decrease in α-adrenoceptor sensitivity. However, these studies can be criticized for fundamental weaknesses in their pharmacological approach (see Introduction Part VII).

We investigated the contribution that altered β-adrenoceptor sensitivity might be making to the progesterone-induced decreased responsiveness to noradrenaline by blockade of β-receptors with

propranolol. Propranolol potentiated noradrenaline only in the HCG + Progesterone-pretreated group and abolished between-group noradrenaline potency differences. This indicated a relatively increased β-adrenoceptor sensitivity of progesterone-pretreated isthmus which has been previously implicated in the reduced potency of noradrenaline with progesterone treatment (Martin et al., 1970; Coutinho et al., 1971; Hodgson and Pauerstein, 1974, 1975). Thus, β-activity changes may be of importance in altered tubal contractility that might be related to the modification of ovum transport.

It is interesting to consider that while β-blockade eliminated between-group differences in noradrenaline ED_{50} values, propranolol did not have such an effect on EF_{50} values for transmural stimulation (greater in HCG + Progesterone than Estrous group). An explanation might be found in the possibility that 1) noradrenaline released from adrenergic nerve endings may have a different access to β-adrenoceptors than exogenous noradrenaline, or 2) the potentiating influence of propranolol on the response of HCG + Progesterone tissues to transmural stimulation may be masked by an inhibition of transmitter release by propranolol.

Tubal contractility is not the absolute regulator of ovum transport. However, altered β-activity is compatible with the existing concepts of modification of ovum transport by progesterone; i.e., reduced responsiveness to noradrenaline with progesterone treatment might decrease the isthmic barrier to transport and result in acceleration of ovum movement through the oviduct.

This early work led us to concentrate on an investigation of oviductal adrenoceptors, particularly β-receptors in Estrous (control)

and HCG + Progesterone-pretreated isthmus.

The inhibitory effects of β-agonists have been clearly demonstrated on rabbit isthmus in vivo (Longley et al., 1968; Howe and Black, 1973). However, in vitro preparations of circular isthmus are less suitable for demonstrating the effects of β-adrenergic stimulation directly, since there is little spontaneous activity and β-stimulation produces little apparent change in tension.

In this study, we chose to look at β-adrenoceptor activity in our in vitro preparations in a system of functional antagonism. Functional antagonism is defined as 'two agents acting on different receptors and having opposite effects in the same effector organ' (Offermeier and Van den Brink, 1974). Hence, we studied the β-inhibitory effect of (±)-isoprenaline on the excitatory response to a cholinergic agent.

In progesterone-treated tissues, isoprenaline inhibited the contractile response to acetylcholine by about 90% while in Estrous tissues, isoprenaline only inhibited the acetylcholine response by about 30%. While this might be interpreted to illustrate increased β-receptor activity in the HCG + Progesterone group, it was also important to eliminate another possibility: that between the different hormone groups there might be some change in the intrinsic capacity of the isthmic smooth muscle to relax. To investigate this possibility, papaverine, a classical non-specific smooth muscle relaxant, was employed. Papaverine exhibited identical dose-response curves in both hormone groups, and thereby demonstrated that the capacities of tissues from both hormone groups to relax were similar. This result, then, offered some support for the hypothesis of altered β-activity between

the groups.

The ID_{50} values for the isoprenaline inhibition of the acetylcholine response determined in this study (Estrus: 3.4×10^{-7}M; HCG + Progesterone: 5.2×10^{-7}M) were in close agreement with those quoted by Hodgson and Pauerstein (1975) for rabbit isthmus (Estrus: 4.5×10^{-7}M; HCG + Progesterone: 5.4×10^{-7}M).

Thus, while the maximum inhibitory effect of isoprenaline was greater in the HCG + Progesterone group than in the Estrous group, the potency of isoprenaline on β-receptors, calculated from normalized curves, did not vary significantly between groups. This indicated that the difference in tissue responses to isoprenaline might reflect a change in number rather than type of receptor. The present study does not answer the question of whether this might be accomplished by an unmasking of, or de novo formation of β-adrenoceptors. It is interesting to recall, however, that progesterone must be administered for 3 days prior to ovulation for its effect on oviduct motility and ovum transport to be realized: this interval could represent the synthesis of new receptors.

Listing adrenergic agonists in their order of potency on adrenoceptors is a classical means of classifying receptor types. Ahlquist (1948) described two distinct potency series on various physiological responses when he first defined α- and β-adrenoceptors. This classification has since been validated particularly through the use of specific α- and β-antagonists. In addition, Ahlquist's work has been extended to encompass the observation that β-adrenoceptors are not identical in all tissues. Lands et al.(1967) listed relative agonist potencies on fatty acid mobilization, cardiac stimulation, broncho-

dilatation and vasodepression to define β-receptor subtypes 1 and 2.

In the comparison of agonists with high and essentially equal efficacies, the relative potencies should be the same for all responses mediated by the same receptor type, regardless of the test preparation, provided that experimental conditions are satisfactory (Furchgott, 1972). This has been illustrated by Furchgott for the potencies of adrenaline, noradrenaline and phenylephrine on α-adrenoceptors in various tissues and species.

In this study, we characterized the α-adrenoceptors in rabbit oviductal isthmus by listing the order of potency of adrenergic agonists in the presence of blockade of catecholamine re-uptake processes and of β-adrenoceptors. The relative potencies of adrenaline, noradrenaline and phenylephrine were similar to those quoted by Furchgott (1972) for α-adrenoceptors in other rabbit tissues and in other species. Thus, the α-adrenoceptor in rabbit oviductal isthmus is identical to that in other systems.

The order of potency for adrenergic agonists on β-receptors of rabbit isthmus ((±)-isoprenaline ≥ (-)-noradrenaline > (-)-adrenaline >> (±)-salbutamol) was simliar to that described by Furchgott (1972) and others (eg., Coleman and Somerville, 1977) for β_1 receptors. Salbutamol, a selective β_2-agonist (Cullum et al., 1969), was much less potent than adrenaline. Not only was the potency of salbutamol reduced, but so too was its maximum inhibitory effect. These findings provided information about the β-adrenoceptor subtype and that it might be β_1 in nature.

In order to further define the nature of the β-adrenoceptor subtype, pA_2 values were calculated for propranolol and practolol,

a specific β_1-antagonist. The pA_x value was defined by Schild (1947) as 'the negative $\log_{10}$ of the molar concentration of an antagonist drug which will reduce the effect of a multiple dose x to that of a single dose'. An important application of pA_x determinations is that receptor types may be identified by means of antagonist actions: for a given agonist-antagonist pair, the same pA_x value on different preparations is indicative of the same receptor type (Arunlakshana and Schild, 1959). The pA_2 values calculated for propranolol and practolol against isoprenaline are comparable to those quoted by Furchgott (1972) for β_1-adrenoceptors in other rabbit tissues.

The pA_2 values for propranolol (Furchgott, 1972) do not adequately distinguish β_1- and β_2-adrenoceptors (β_1: 8.8; β_2: 6.9, 9.0). The pA_2 value determined for propranolol in this study (8.3) was of the same order as these published results. However, pA_2 values for practolol, a specific β_1-antagonist, do illustrate differences between β-adrenoceptor subtypes (β_1: 6.9; β_2: 3.9, >3.5), in that such low pA_2 values for the β_2-receptor subtype indicate a largely non-specific antagonism. The pA_2 value for practolol determined in this study (5.3) was comparable to the values for the β_1-receptor type.

Thus, the calculated pA_2 values for the propranolol and practolol antagonism of the isoprenaline inhibitory effect demonstrate that the β-adrenoceptor subtype in the circular muscle of rabbit isthmus (HCG + Progesterone) is largely β_1 in nature. β-Adrenoceptors in other muscle layers or in other species have not been sufficiently studied to determine their β-receptor subtype.

II Summary

The major findings of the present study may be summarized in the

following points:

1) α- and β-Adrenoceptors are present in the circular smooth muscle of rabbit oviductal isthmus. α-Adrenoceptors predominate since the tissue response to adrenaline, noradrenaline and transmural stimulation was always a contraction which was blocked by phenoxybenzamine or phentolamine. The character of the α-adrenoceptor in rabbit oviductal isthmus is similar to that in other tissues and species.

2) Noradrenaline exhibits a decreased potency in oviductal isthmus following the HCG + Progesterone-pretreatment which is known to cause the acceleration of ovum transport.

3) Catecholamine re-uptake processes and α-adrenoceptor sensitivity are not considered to contribute to contractility changes which might be related to modification of ovum transport by hormones.

4) A relatively increased β-adrenoceptor activity is proposed for HCG + Progesterone-pretreated rabbit isthmus.

5) The β-adrenoceptor of rabbit oviductal isthmus was characterized by agonist potency studies and antagonist pA_2 determinations to be largely β_1 in nature.

The findings of this study contribute to the growing body of evidence that the modification of ovum transport by sex hormones is mediated by oviductal adrenergic mechanisms. The proposal of increased β-adrenoceptor activity in HCG + Progesterone-pretreated rabbit isthmus may be satisfactorily incorporated into the existing concept of modification of ovum transport by progesterone: the reduced responsiveness to noradrenaline following progesterone pretreatment might decrease the isthmic barrier to transport and result

in acceleration of ovum movement through the rabbit oviduct.

III Future Work

Much work remains to be completed before a satisfactory analysis of the relationships between steroid hormones, the adrenergic nervous system and oviduct physiology is achieved. This study has contributed a small amount of knowledge towards solving this largely unanswered problem. Perhaps more importantly, it has raised questions which may form the basis for future research in this area.

An increase in β-adrenoceptor activity has been implicated in the reduced responsiveness to noradrenaline of progesterone-pretreated rabbit isthmus. It would be valuable to assess whether this is the result of increased numbers of β-receptors. This might involve the labelling of oviductal β-adrenoceptors with labelled antagonists, or alternatively, might involve monitoring the effect of protein synthesis inhibitors on the modification of β-activity by progesterone.

Hormonally-dependent adrenoceptor sensitivity changes have been well documented, at least with reference to effector oviductal smooth muscle. It would be of interest to investigate whether there is also a presynaptic receptor change which would influence the release of noradrenaline from adrenergic nerve endings.

Although advanced techniques are employed in the study of the electrical and mechanical activity of the oviduct, there are few good investigations of the cellular mechanisms mediating the actions of catecholamines in the oviduct. Therefore, it might be of considerable interest to study the effect of hormones on cyclic AMP production following β-adrenergic stimulation or on intracellular Ca^{++} sinks or

transport mechanisms.

Finally, *in vitro* findings must be correlated with *in vivo* observations, and preferably in humans, before research on the interrelationship of ovarian hormones and oviductal adrenergic mechanisms in the modification of ovum transport may ultimately contribute to fertility regulation.

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APPENDIX

TABLE V Effect of Various Adrenergic Agents on the Potency (ED_{50} ± S.E.M. (n)) of Adrenergic Agonists in Estrous Oviductal Isthmus.

	ED_{50} ± S.E.M. (n)		
Agent	Adrenaline	Noradrenaline	Phenylephrine
Control	4.22×10^{-7} ± 0.46 (9)	3.92×10^{-6} ± 0.23 (9)	3.50×10^{-6} ± 0.56 (11)
Cocaine	2.13×10^{-7} ± 0.31 (8)	5.05×10^{-7} ± 1.53 (8)	3.92×10^{-6} ± 1.04 (7)
Hydrocortisone	3.86×10^{-7} ± 2.25 (5)	4.32×10^{-6} ± 0.89 (5)	4.47×10^{-6} ± 0.65 (5)
Propranolol	3.54×10^{-7} ± 1.13 (5)	5.83×10^{-6} ± 1.67 (5)	5.93×10^{-6} ± 1.43 (6)
Cocaine + Hydrocortisone	1.54×10^{-7} ± 0.47 (7)	4.18×10^{-7} ± 0.71 (6)	4.23×10^{-6} ± 1.41 (5)
Cocaine + Hydrocortisone + Propranolol	5.01×10^{-7} ± 1.31 (5)	6.84×10^{-7} ± 2.53 (5)	3.75×10^{-6} ± 2.01 (5)

TABLE VI Effect of Various Adrenergic Agents on the Potency (ED_{50} ± S.E.M. (n)) of Adrenergic Agonists in HCG + Progesterone-pretreated Oviductal Isthmus.

Agent	ED_{50} ± S.E.M. (n) Adrenaline	Noradrenaline	Phenylephrine
Control	$6.81 \times 10^{-7} \pm 1.32$ (9)	$21.69 \times 10^{-6} \pm 3.90$ (11)	$5.13 \times 10^{-6} \pm 1.0$ (9)
Cocaine	$4.23 \times 10^{-7} \pm 0.78$ (10)	$5.14 \times 10^{-6} \pm 1.18$ (12)	$8.77 \times 10^{-6} \pm 2.50$ (11)
Hydrocortisone	$8.68 \times 10^{-7} \pm 3.45$ (6)	$24.24 \times 10^{-6} \pm 6.12$ (5)	$5.30 \times 10^{-6} \pm 1.00$ (5)
Propranolol	$7.27 \times 10^{-7} \pm 1.94$ (6)	$7.95 \times 10^{-6} \pm 2.00$ (5)	$5.45 \times 10^{-6} \pm 2.50$ (5)
Cocaine + Hydrocortisone	$6.69 \times 10^{-7} \pm 2.40$ (5)	$7.20 \times 10^{-6} \pm 2.08$ (5)	$6.54 \times 10^{-6} \pm 0.23$ (5)
Cocaine + Hydrocortisone + Propranolol	$6.76 \times 10^{-7} \pm 1.59$ (5)	$6.32 \times 10^{-6} \pm 0.82$ (5)	$10.73 \times 10^{-6} \pm 3.61$ (4)

TABLE VII Effect of Various Adrenergic Agents on the Potency (ED_{50} ± S.E.M. (n)) of Adrenergic Agonists in HCG + Estrogen-treated Oviductal Isthmus.

Agent	ED_{50} ± S.E.M. (n) Adrenaline	Noradrenaline	Phenylephrine
Control	$5.55 \times 10^{-7} \pm 0.86$ (12)	$5.63 \times 10^{-6} \pm 0.77$ (12)	$6.16 \times 10^{-6} \pm 1.51$ (10)
Cocaine	$3.97 \times 10^{-7} \pm 1.55$ (9)	$1.49 \times 10^{-6} \pm 0.48$ (10)	$9.90 \times 10^{-6} \pm 2.93$ (9)
Hydrocortisone	$8.42 \times 10^{-7} \pm 2.59$ (5)	$9.17 \times 10^{-6} \pm 1.64$ (5)	$7.06 \times 10^{-6} \pm 0.91$ (5)
Propranolol	$4.35 \times 10^{-7} \pm 0.55$ (5)	$5.32 \times 10^{-6} \pm 1.18$ (6)	$9.06 \times 10^{-6} \pm 2.76$ (5)
Cocaine + Hydrocortisone	$1.87 \times 10^{-7} \pm 0.81$ (6)	$1.16 \times 10^{-6} \pm 0.33$ (5)	$7.08 \times 10^{-6} \pm 1.57$ (6)
Cocaine + Hydrocortisone + Propranolol	$4.74 \times 10^{-7} \pm 1.80$ (4)	$1.15 \times 10^{-6} \pm 0.34$ (5)	$6.99 \times 10^{-6} \pm 1.91$ (5)